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EVIDENCE FOR CONTROL OF NYSTAGMIC HABITUATION BY FOLIUM TUBER VERMIS AND FASTIGIAL NUCLEI

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EVIDENCE FOR CONTROL OF NYSTAGMIC HABITUATION BY FOLIUM TUBER VERMIS AND FASTIGIAL NUCLEI

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CONTENTS

Although habituation of vestibular nystagmus has been extensively studied, the primary neural elements underlying this process have not been delineated

There is no clear-cut answer as to whether habituation of nystagmus is due to interactions between brain stem and cortical levels, or takes place as a function of any specific neural structure In fact, there is even disagreement as to the site of origin of the fast and slow phase According to Spiegel & Price (1942) the vestibular nuclei may produce both components Gernandt (1964) is of the opinion that the labyrinth initiates the slow phase of nystagmus, but the center for control is in the vestibular nuclei Szentágothai (1943, 1950) has proposed the mesencephalic reticular formation as the center for control of horizontal eye movements.

Experimentation with barbiturate anesthetics has tended to support the view that the mesencephalic reticular formation is involved with the habituation process. This structure is known to be particularly sensitive to barbiturates, and animals do not habituate when stimulated under barbiturate anesthesia (Fearing & Mowrer 1934) However it is a well known clinical fact that the cerebellum also is highly sensitive to barbiturate anesthetics. Halstead (1935 p 115) found that birds with cerebellar lesions habituated less than normal control animals. Although the effects of barbiturate anesthetics on habituation have been examined, determining the sites of action of the barbiturate remains a problem

Effects of Arousal Level

One of the many problems in the investigation of the habituation of vestibular nystagmus has been adequate control of arousal. In 1914 Guttich reported that repeated exposure to the Bárány rotation test usually results in a marked decrement in nystagmus duration he attributed this to a *decline in the general nervous tension* which *subjects usually display* during the first few trials of the test, rather than to any actual decrement in the strength of the reflex (Mowrer 1934 *b* p 10) Wendt (1951 p 1215) later emphasized the importance of maintaining "an environment directed orientation" in vestibular studies employing both animals and man

The more recent methods of controlling arousal level have been fairly well standardized With humans, mental arithmetic has been used in an attempt to maintain a state of alertness (Collins, Crampton & Posner 1961 Collins, 1962 Collins, Guedry & Posner 1962) Although amphetamines have been used with humans (Collins & Poe, 1962) this technique has been

more commonly employed with animals (Crampton, 1961 1964) There is some question as to the appropriateness of this method since with the high dosage levels used, some animals are ataxic and appear to have vestibulo-cerebellar impairment.

Stimulus Alternatives

A potential error in the results of vestibular experiments has been the restriction in the choice of a stimulus. With the exception of a very few studies (e.g Wendt, 1964 Jones, 1965) constant angular acceleration has been used as the stimulus the subject is rotated at continuously increasing speed in a given direction to a pre-determined terminal velocity The stimulus lasts only for the brief period of acceleration, and it is usually necessary to employ a five minute inter trial interval to avoid interactive effects of secondary nystagmus. Also, long duration constant angular acceleration is an unusual stimulus, in that most voluntary head movements involve a short acceleration followed by an immediate deceleration (Wendt, 1951) In the present study a stimulus was used which approximates normal head movements. The subject was continuously oscillated in the horizontal plane about the vertical axis Sinusoidal oscillation offers distinct methodological advantages since the subject is stimulated continuously and there is no need for long intertrial intervals. Furthermore since the stimulus can be analyzed in degrees of displacement/unit time, sinusoidal stimulation permits closer correlation of the stimulus with evoked potentials, and allows for within subjects controls such as responses to auditory or electrical stimulation

Neurophysiology and Anatomy

Cray (1926, p 321) attempted to determine if anatomical evidence supported the theory of reciprocal innervation of the two halves of the body and further whether mediation was by the vestibular nuclei and their fiber tracts His conclusion was that the major influence seemed to be via the uncinate *fasciculus (hooked bundle of Russell) from the fastigial to the* vestibular nuclei "This connection suggests that the vermian cortex, under afferent stimulation from the muscle spindles, may normally exercise a tonic influence on the vestibular nuclei This view would explain forced movements and disturbances of equilibrium after median cerebellar injuries and stimulations. It would account for decerebrate rigidity' and for the fact that this rigidity can be inhibited by stimulation of the cerebellar cortex" (Cray 1926 p 353) In addition, Devito *et al* (1965 p 253) found that cerebellar polarization not only inhibited decerebrate rigidity but also blocked spike discharges from the lateral vestibular nucleus Gernandt & Gilman (1959) Cohen *et al* (1958) and Fernández & Fredrickson (1964) have also shown with stimulation and ablation techniques the inhibitory influence of the cerebellum upon vestibular activity

Cohen *et al* (1965 p 157) were able to induce a wide range of con

jugate eye movements by cerebellar stimulation in the cat. Except for minor differences, these responses were very similar to those obtained from stimulation of the semicircular canals. They concluded that the cerebellum, when stimulated, has a topographic representation for eye movements. Electrical stimulation of the tuber vermis and fastigial nuclei led to conjugate horizontal eye movements, whereas in the more lateral and dorsal structures, it led to vertical eye movements. These physiological findings clearly support Gray's conclusions based on anatomy

Generally physiological and psychological investigations of the vestibular and cerebellar systems have employed one of three major methods electrical recording electrical stimulation or ablation. The present research was an attempt to bring these three methods together to investigate the functional relationships between habituation to sinusoidal vestibular stimulation and the cerebellar posterior vermis, fastigial nuclei, uncinate fasciculus, mesencephalic reticular formation and centrum medianum

METHODS

Subjects

Fifteen cats—10 females, three males and two castrated males ranging in weight from 6 to 8 lbs. were used

Apparatus for Stimulation

Sinusoidal stimulation was provided by a turntable which could be mechanically oscillated 30 around the vertical axis. The turntable consisted of a platform approximately 2 1/ × 4 1/2 mounted on a 360 bearing system Power was provided by a 1/2-horsepower 115 vac motor The output from the motor was delivered by a V belt drive to a 7.5 1 gear box In addition to increasing the torque, the gear box allowed for a 90 shift in output power from the horizontal to the vertical axis Frequency of oscillation was kept constant at .33 cps. The turntable was housed in a light tight room *S* was separated from the electronic stimulating and recording equipment

The sinusoidal stimulus was converted to an electrical impulse by mounting a sine-cosine resolver potentiometer (clarostat SG 42-070 10K) at the center of rotation Power to the potentiometer was provided by a 6 V D C. battery The signal was led out through slip rings and displayed on the ink writer

Calibration Lights

A row of three lights, 4 apart was placed approximately 10 in front of the animal at eye level (10 between lights) to permit calibrated eye movements. The three lights could be individually controlled for brightness.

Tone Generator

Seventy to 80 db (SPL) tones of 400 500 and 900 cycles were generated by amplifying the output from a Grass S4G stimulator with a Heathkit Model EA-3 amplifier The signal from the amplifier was transmitted to a 5 speaker located approximately 3 from the *S*

Restraint Apparatus

Pilot work with the method of animal restraint developed by Henriksson Fernandez and Kohut (1961) proved the technique to be inadequate for artifact free recording The Ss were continually licking at the wire through their teeth, leading to artifacts on the EEG recordings. Consequently the cats were restra n l in w oden box adjustable to the size of the animal

All animals had head pedestals that were firmly anchored both in the frontal sinus cavity and over the skull A headholder consisting of a clamp arrangement was designed to hold the animal firmly by this pedestal This technique proved eminently satisfactory in that it provided firm restraint and recording essentially free of licking movement, and other artifacts.

Recording Apparatus

All EOG and electrophysiological potentials were recorded on an Offner Type R Dynograph Sinusoidal oscillation was monitored with a 50K ohm resistor across the input cable and slip ring assembly Since both the resistor and cable act as transducers, any vibration or nonsymmetrical cable sway would be detected by the differential amplifiers. Periodic checks throughout the experiment insured maintenance of low noise levels (2–4 μV input shorted)

Electrical Stimulator

Sub-cortical electrical stimulation was delivered from either a Grass S4G constant voltage source or a constant current device (Model 7150) manufactured by Nuclear Chicago, Chicago, Illinois.

Recording Electrodes

Three different types of electrodes were used

1) Electro-oculographic (EOG) electrodes. These consisted of a 4" strip of no 30 gauge stainless steel, formvar coated wire that was welded or soldered to a 000–120 × 1/" stainless steel screw for permanent attachment to the skull.

2) Silver ball electrodes for surface recording Twenty-eight gauge pure silver wire was cut into 4"–5" lengths, and then heated in a gas or alcohol flame until a small ball (.5–1 0 mm diameter) was formed. Each wire was then painted with four five coats of insulx. Cortical electrode placement is shown in Fig 6

3) Bipolar electrodes for deep brain implantation. Thirty gauge no. 304 stainless steel formvar coated wire was used in the construction of these electrodes. A pair of these wires was aligned with a longitudinal tip separation of 1 0–1.5 mm The wires were fastened together by applying four–five coats of insulx, and allowing them to dry for at least 24 hours.

Preoperative Training

Prior to surgery all *Ss* were given a week's training (one session/day) in the restraint apparatus to adapt them to the restraint

Surgery

Surgery was performed under sterile conditions. Following surgery all *Ss* received 300 000 units (intramuscularly) of crysticillin, an antibiotic. The *Ss* were anesthetized with intra peritoneal injections of sodium pento-

barbital (60 mg/cc solution Nembutal) The first six *Ss* were implanted using electrophysiological monitoring. However variability in the anesthetic levels produced extreme variation in the electrical activity of the brain and made it impossible to identify true evoked responses. Since the electrodes were almost always set at the stereotaxic coordinates, monitoring was discontinued in subsequent operations

After placing *S* in a stereotaxic instrument, a midline incision was made from the frontal sinus to the region of the first cervical vertebrae At the time of the initial incision while the bone was still translucent, it was possible to visualize the frontal air sinus, and a mark was made over this region After rongeuring away the bone the sinus epithelium was aspirated and all sinus passages blocked with sterile bone wax. A no $^1/_2$ dental drill was used to bore into the medial surface of each bony orbit to permit placement of the EOG electrode A small shelf of bone was isolated on the midline, and the reference electrode for the eyes was placed at this point The sinus was allowed to dry and then filled with dental acrylic

The neck muscles were reflected from the lambdoidal crest, and the bone overlying the cerebellar vermis and pyramis exposed Silver ball electrodes were placed at various locations on the surface of this area Deep brain electrodes were implanted stereotaxically to avoid the tentorium electrodes intended for the fastigial and medial vestibular nuclei were inclined at an angle of 30 Electrodes were implanted in the following sites anterior ectosylvian gyrus, nucleus centrum medianum (centralis centralis of Snider & Neimar 1961) lateral geniculate body mesencephalic reticular formation medial vestibular nucleus, nucleus fastigius, cerebellar tuber vermis, cerebellar pyramis, and striate cortex.

All electrode leads were anchored to the skull using dental acrylic A layer of acrylic was then built up along the midline and a 20 pin miniature female plug was embedded in place The leads were then led across the skull and soldered to the wires on the plug The pedestal was formed by building acrylic around the wires and plug This also provided insulation of individual wires. The wound edges were brought together around the pedestal by means of gut sutures.

In two *Ss*, cerebellar ablations were performed by means of subpial aspiration In one surgery was performed 2 $^1/_2$ months after the *S* had been implanted with recording electrodes The bone was then rongeured away and a lesion of the tuber vermis performed In the second case the lesion was made (tuber vermis) and surface recording electrodes implanted during the same operation

Postoperative Procedure

Following surgery the *Ss* were allowed to recover for four-five days, and then were retrained in the restraint box The head clamp was placed around the pedestal and a male plug attached The *S* was kept in the box for 15-20 minutes and then released and rewarded A minimum of 10 days

elapsed before *Ss* were tested in the actual experimental situation. All *Ss* were tested on a random schedule with at least one day elapsing between sessions. The experiment involved seven different conditions.

1) Calibration of EOG—*S* was placed in the dark and the calibration lights were turned on and off in sequence. A stimulus mark was made on the EEG during the period in which the light was on.

2) Zero-degree condition—*S* was oscillated with the head in a level plane.

3) Nineteen degree condition—*S* was oscillated with the head inclined forward at this angle.

4) Auditory alert (dishabituation)—Tones of various frequencies (400 500 and 900 cps) were sounded during the period in which the *S* appeared to have habituated.

5) Mesencephalic reticular stimulation—*Ss* were stimulated electrically at various times during the recording session.

6) Auditory control—*Ss* were suspended on a platform and the turntable oscillated beneath them.

7) Nembutal—Before perfusion, *Ss* were oscillated under varying levels of sodium pentobarbital and one *S* under chloralose. Stimulation of the reticular formation, centrum medianum and fastigial nuclei was also used under this condition.

Histology

At the conclusion of the study all *Ss* were given a lethal dose of sodium pentobarbital, and perfused through the heart with 10% formalin solution in saline. Brain tissue was sectioned (40 microns) using the frozen method, and every tenth and eleventh section was alternately stained with cresyl violet or iron hematoxylin.

General Recording Procedure

Pilot work with humans (Wolfe & Wendt, 1966) indicates that during the first 10–15 minutes in the dark, there is no significant change in the amplitude of the corneo-retinal potential. Since no information is available with respect to the effects of dark-adaptation on the corneo-retinal potential in the cat, an attempt was made to keep conditions constant for all *Ss*. The *Ss* were exposed to room level illumination for 10–15 minutes prior to each recording session. After being placed in the restraint box, a record (1–5 min) was taken from each *S* before stimulus onset, while the animal was voluntarily looking around the room.

RESULTS

Behavioral observations which may be relevant to the results have been included

The results of electrical stimulation during oscillation are organized into unanesthetized and anesthetized conditions. Tables 1 and 2 are summaries of EOG responses to electrical stimulation under both conditions. Finally the effects of lesions of the tuber vermis are considered with respect to their immediate and long term effects.

Behavioral Observations

After recovery from the anesthesia (12–24 hours) *Ss* typically displayed hypermetria of the hind leg ipsilateral to the fastigial electrode site If *Ss* were pushed lightly they fell to this side Placing reflexes of the front legs were intact in all *Ss*, but, for the first three–five days postoperatively placing of the ipsilateral hind leg (either right or left depending on implant) was partially impaired

S 428 inadvertently was given an injection of heparin (40 cc) prior to surgery Subsequently the animal lost a great deal of blood and sustained varying subcortical damage with electrode implantation This animal had more pronounced signs of cerebellar damage Both hind legs were "stiff" and the animal was unable to stand for 48 hours.

No attempt was made to check righting reflexes by dropping the animals, since this could have damaged their electrode pedestals. However checks for postural nystagmus were made one–two days postoperatively by placing each animal on its sides and back and examining its eyes. Although an occasional beat was seen in some of the animals, there was nothing which could be considered abnormal Two *Ss* (*S* 394 and *S* 402) had a transient (two–three day) strabismus of the eyes, but other than this, none of the animals showed oculomotor deficits.

At the beginning of the first recording session *Ss* were highly emotional as reflected in pupillary dilation As a result, with the first stimulus onset most of them struggled for a few seconds leading to artifacts on the EEG With the exception of S 388, there was little or no struggling during subsequent recording sessions.

Recording of the EOG

Calibration In cats, the amplitude of the EOG for a known eye displacement has not been precisely determined Since cats show few voluntary eye movements in the dark an attempt was made to obtain a calibration

by having them look at a light (a novel stimulus) This approach proved fairly successful In the case of *S* 409 (Fig 1) the first two responses were almost identical in amplitude The drift of the EOG back to the baseline is due to the 3-second time constant indicating that *S* maintains a fairly good fixation of the light and holds this position when the light is turned off As soon as the EOG returned to the baseline the next light in the series (10 to the right) was turned on. The next two responses were not as discrete. Therefore, the experimenter must use some judgment as to which of the measures to use in the final calculation. Animal 400 (Fig. 1) reflected a similar response pattern It was found that the amplitude of the EOG was fairly consistent for a given eye displacement For example, the computed displacement per degree of eye movement was .85 mm for *S* 409 and .90 mm in the case of *S* 400 The average for all *Ss* was 92 mm/degree Periodic calibration checks showed no significant changes. As with any other calibration, this value may be influenced by electrode placement, particularly with acute needle electrodes, and therefore should be computed before each experimental session.

Habituation to Oscillation

On the first trial, before habituation, all animals excepting *S* 428 displayed a normal nystagmus (compensatory eye movement with fast phases in the direction of displacement) As habituation occurred systematic changes in responses were evident both within and between trials. Two quite different changes were noted.

The first change is best characterized as an almost complete loss of the fast phase of nystagmus, with no change in the amount of compensatory slow phase Inspection of Fig 2 illustrates the form of the response in an habituated animal Had visual surrounds been available to the cat, this change would have been in the direction of greater functional adequacy i.e maintenance of visual fixation.

The second change involves the appearance of episodes when normal compensatory eye movements do not occur for varying periods of time In the intact, unanesthetized cat, subjected to 30 of oscillation these phase shifts normally occur as a slow drift of the eyes (often somewhat uneven in rate) toward the direction of rotation. Thus, they are opposite to the reflex compensatory response required by the oscillation and for maintenance of visual fixation. A curious feature of these episodes is that they most commonly occur not at the turning points of the oscillatory stimulus, but during the intermediate periods when the velocity of motion of the platform (and of the unhabituated eye movement) is greatest. At the turn, and just before and after the turn, normal reflex compensatory eye movements usually occur

One cannot make the generalization that during the episodes of loss of compensatory movements the eyes always drift toward the direction of

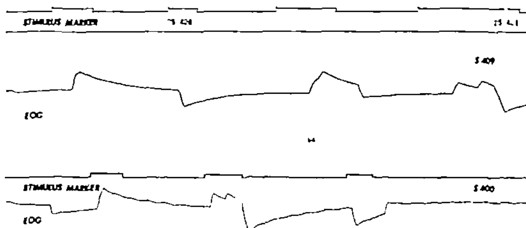


FIG. 1 Calibration f cat EOG by lunt ry ey mo ments. X = 500 μv/cm, 25 mm/sec

F- 20 CPS
EOG
L MF
NOD
R MRF
R VR
L AE
1CM 1SEC
R STRIATE

FIG. 2. H bitual on ecord from S 400 Not almost complet bsence of f l phases. EOG X = 500 m, 25 mm sec

F-33 CPS

EOG

NOD

M UNF

F-33 CPS

EOG

1SEC 1CM

MNF

Fig 3. Bottom record show drift f yes past turn on third cycle altern tions i MNF from HVS t LVF ppea t be rel ted to left-right reversals. Top record reflect l rge monophas ic p kes from the M UNF EOG X = 400 μ cm, ll thers 50 μ m, 250 mm/sec

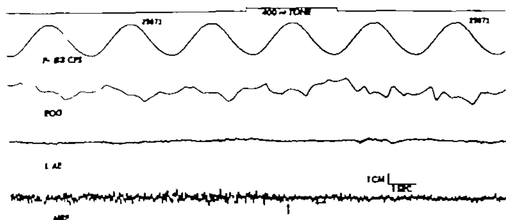


Fig. 4 Effect f dishabit ting stim lus. Not change in MRF from HVS t LVF nd ncrease l ten ity f ystagmic response EOG 400 cm, ll others 50 μ m, 25 mm sec

rotation. Sometimes a drift continues past the point of the turn (see Fig 3)

Another completely different change is seen for the so-called "unilateral" habituation found after cerebellar lesions (see below p 27) The eyes do not drift, but maintain essentially a fixed position (see Fig 20)

These phenomena in the cat are in some ways the same and in some *ways different from those reported by Wendt (1951 1965) for man and* for monkey They are similar in that the eyes sometimes drift toward the direction of head movement and that this motion is often tremulous. They are different in that in man and monkey the periods of non-compensatory eye movement are usually continuous, whereas in cats they are usually not present during the turns.

Effects of a Dishabituating Stimulus

After the animals had habituated to the vestibular stimulus, a brief tone (2–5 sec.) led to an almost immediate increase in the frequency or amplitude of the vestibular nystagmus. This dishabituation was also apparent with other electrophysiological measures

1) The mesencephalic reticular formation (MRF) changed from a pattern of high voltage slow (HVS) to low voltage fast (LVF) activity (Fig 4)

2) In all *Ss*, there were changes in the infraslow potentials from the cerebellar cortex. This was reflected as either an increase in amplitude or a disruption in period of the IFSPs.

The auditory stimulus typically caused a very brief dishabituation which only outlasted the tone for a few seconds. With a few repetitions of the tone, *Ss* soon became habituated to it. As even with changes in frequency there was little or no effect on the vestibular response

Conditioning of Fast Phase

Some evidence for conditioning was obtained in the auditory control situation (animal suspended above the apparatus) These data agree with those of Wendt (1930 *b*) In response to the sound of the drive system, a conditioned stimulus, one sees only anticipatory responses. The Ss showed no eye-movements which could be interpreted as the slow phase of nystagmus Typically a fast phase was seen in response to stimulus onset Sometimes, these were in the direction of oscillation Throughout the trial period *Ss* showed random rapid eye movements. The record from *S* 400 (Fig. 5) shows some of the neural responses that may be involved in the conditioned anticipatory response If one looks closely at the record, it can be seen that the first fast phase was preceded by an increase in the potential from the cerebellar vermis and the right fastigial nucleus. This was followed by a diphasic spike in the left medial vestibular nucleus which was almost simultaneous with the eye movement This animal had received six trials distributed over a period of 21 days prior to the auditory control trial

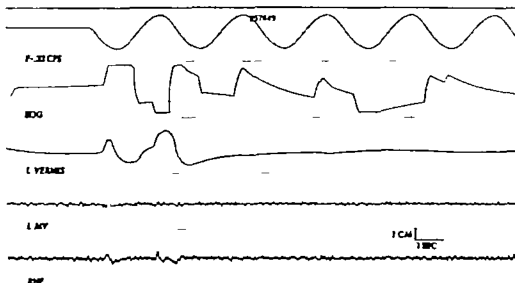


FIG. 6 Auditory control trial. With stimulus onset, increase in potential from L vermis 1 followed by RNF LMV and ey m me t t left. EOG 500 μ cm, L ermis 100 μ cm, all others 50 μv cm, 25 mm sec

Cortical Potentials

In the present study infraslow potential changes (IFSPC) were recorded from various regions of the tuber vermis of the cerebellum (Fig 6 shows electrode placement) Records were obtained with silver ball electrodes and recorded using a 1 or 3-sec time constant. Infraslow oscillations are sinusoidal in form, either with a period of 7–8 sec (A rhythm) and an amplitude of 0.3–0.8 mv or with a period of 0.2–2 min (B rhythm) and an amplitude of 0.5–1.5 mv there are also intermediate values (Aladjalova, 1964 p 1) Infraslow potentials appeared in all animals before stimulus onset Fig 7 indicates a few of the various forms of these potentials prior to stimulus onset Both frequency and amplitude varied as a function of electrode placement and subject The range was from 200–800 μv with a period of 7–10 sec., approximating the A rhythm.

With stimulus onset there was an increase in both the frequency and amplitude of the infraslow potentials. In some cases, changes which appeared to be related to the eye movement responses were superimposed on the slower potential The potential from the vermis of *S* 328 (Fig 8) on the first trial reflects an almost perfect correlation with the EOG. If one aligns the peaks of the vermal response with the eye movements, it can be seen that, when the cerebellar potential reaches its maximum, there is a reversal in the direction of nystagmus As the animal s eye movement response habituated (decrement in amplitude and decrease in the number of fast phases) there was a corresponding decrease in the amplitude of the IFSP and in most *Ss*, an increase in the period of this sinusoidal

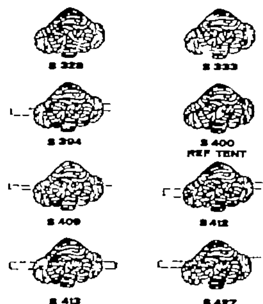


FIG. 6 Cerebella cortical lectrode loci. Commo reference l indicated. All lectrodes were .5–1.0 mm sil er ball

wave This dramatic reduction in amplitude was maintained as long as the animal was periodically stimulated (every three–seven days) In the case of *S* 328, a period of 15 days was allowed to elapse between trials. As can be seen in Fig 9 the IFSP was re-established and in this *S* was higher in amplitude than on the first day of exposure The *S* also displayed a normal (non habituated) nystagmic response which again, was more vigorous than the original response on day 1

In some of the earlier operations, recording electrodes were implanted in the bone overlying the cerebellar cortex. In an attempt to obtain higher level and "more accurate signals from this region the last six *S*s were implanted with cerebellar leads placed directly on the dura. This led to an increase in amplitude and better definition of the vermal response Figs. 10 and 11 show more clearly the relationship between the cerebellar response and the eye movement reversals. In many cases, the vermal potential increased to a maximum just prior to, or almost simultaneously with changes in the EOG In some instances, monophasic and diphasic spikes in the fa tigial and medial vestibular nuclei were apparent at the peaks of the vermal response As the data indicate the left and right vermal responses are not the same Since both records from the vermis were taken across a common reference (see Fig. 6) it is possible to localize the response to a specific recording electrode Furthermore, the difference between the two responses helps rule out the probability of eye-movement artifact

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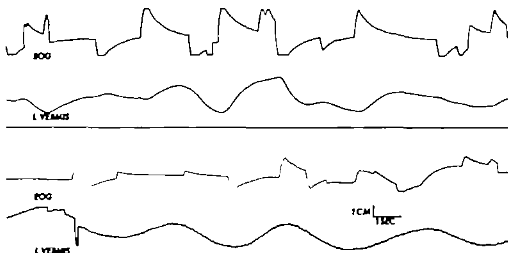


FIG. 7 I frasl w potential from cerebella vermis. Recorded before stimulu nset. EOG 500 μ /cm ermi 100 μ cm (3 sec T C.) 25 mm sec

EEG Changes Associated with Voluntary Eye Movements

Potentials from the visual radiations did not reflect a 1 1 correspondence with rapid eye movements as has been found in some primates (Perachlo 1966) Although this structure responded to changes in eye position, the response was not discrete but usually a phasic burst of activity Cortical leads over primary visual cortex, showed a large amplitude (200–300 μv) monophasic spike which was correlated almost 1 1 with the fast phase of voluntary eye movement (Fig 12) Fig 12 also shows that this potential is not due to changes in neck muscle potential, since the EMG amplitude in this case is only approximately 20 microvolts and does not reflect a temporal relationship with the EOG. Electrical stimulation of the visual radiations (10/sec 3 volts, 1 msec.) led to a cortical response but did not cause eye movements in the one subject stimulated

Sub-Cortical Potentials

Histological study revealed that three of the animals had electrode placements exactly between the two fastigial nuclei and a fourth *S* with an electrode on the midline in the dorsal uncinate fasciculus (see Table 3) Those animals with the midline fastigial electrodes showed an alternation in potential from HVS to LVF activity which appeared to be related to the reversal points of the eyes (Fig 3) The responses from the uncinate fasciculus in *S* XYZ were more discrete and easier to identify In many cases, they were bracketed by large monophasic spikes. In all animals these changes came at two intervals 37–38 mm (1.5 sec) and 45–47 mm (1 7 sec) the former period being equal to one-half of the oscillation

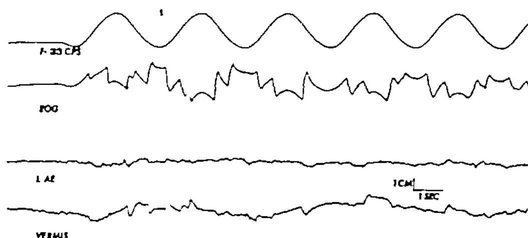


FIG. 8. Record f EOG and vermal pot tial taken on first trial Peaks f ermal potential correlat 1 1 with EOG reversals. EOG 500 μ /cm, LAE d vermis 100 μv/cm

F-33 CPS
EOG
R MF
L UMF
R VR
VERMUS
1SEC
1CM
L MRF

FIG. 9 Cha ges i EOG d ermal respo ses fter 16 da period betwee tri ls. EOG 500 μ m, ermi 100 cm, ll thers 50 μ cm.

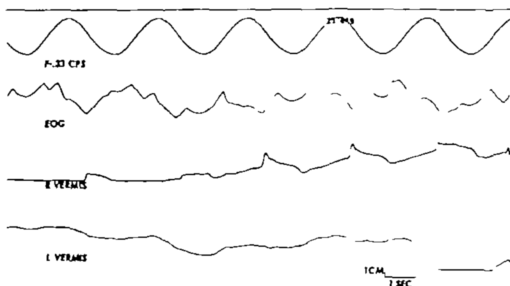


FIG. 10 S 427 Pote tial from the R ermis reaches m ximum just prior t the EOG reversal t the left. The small denotes adjustment of the zero point by E, nce the pen was at n lectrical limit prio t this. EOG 500 μ cm, vermis 100 μv cm, 25 mm/sec

F-.03 CPS
EOG
L VERMIS
R AMV
L MF
CM
1CM
1SEC
R VERMIS

FIG 11 S 400 Ag in. L ermal potential ncreases t max. prior t EOG rev rsal t left. Note alternations RM\ from LVF t HVS t peaks of the ermal potential. Left nd right are onl used t denote lectrode location (see Fig. 6) EOG 500 μ /cm, verml 100 μ cm, ll others 50 cm, 25 mm sec.

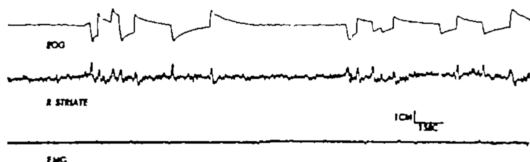


FIG 12 S 400 Relationship of striate potential to EOG Amplitude of striate response appears to be related to velocity and not amplitude of the fast phase; striate potential may possibly be related to saccadic suppression. EOG 500 μ / cm, striate 100 μ /cm, EMG 20 μ cm, 25 mm/sec

cycle This same patterning was also seen in the medial vestibular nucleus (Fig 11) and nucleus fastigius (Fig 13)

All leads showed a change in activity with stimulus onset The fastigial and medial vestibular nuclei reflected the greatest change in activity and was often correlated with changes in the EOG

Electrical Brain Stimulation During Oscillation

Stimulation in the unanesthetized S No attempts were made to stimulate the fastigial nuclei in the unanesthetized animal since numerous studies indicate that seizures often result (Dow & Moruzzi 1958) Histological study showed that two of the Ss had placement in the superior colliculus and another animal had electrodes in the fornix commissure One would expect that stimulation in these sites would lead to arousal of the animal and in fact changes in the nystagmic response reflected the anticipated alerting

S 42 (Fig 14) had a good placement in the MFR In this case a stimulus of only 10 microamps caused an increase in intensity of the nystagmic response and a simultaneous change in the vermal potential Fig 14 substantiates the fact that this cerebellar potential leads the eye-movement reversal with stimulus offset there also was an increase in frequency and amplitude of the response

S 328 showed the most dramatic change in response to MRF stimulation (Fig 15) Based on voltage across a 1k resistor it was estimated that the stimulus was approximately 250 microamps However stimulus duration was only 1 msec Prior to stimulation, S had habituated to the vestibular stimulus and the IFSP had almost returned to the baseline With stimulus offset there was an increase in the amplitude of the IFSP but little or no

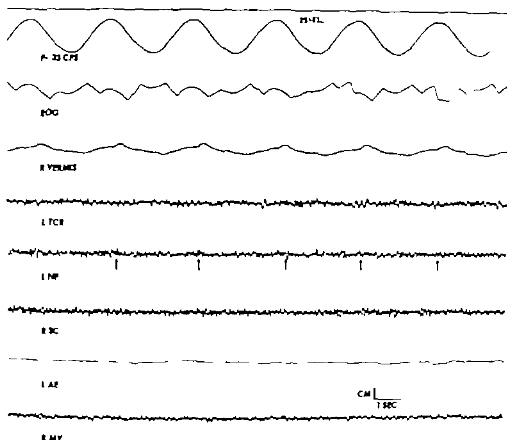


FIG. 13. Arrows denot lternati ns in potential from cleus fastigi t the peaks f the vermal response EOG 500 μ cm, ermi d AE 100 m, all others 50 μ cm, 25 mm/sec

change in the nystagmic response However 5 /₂ sec. after the stimulus, there was a spontaneous discharge in the fastigial nucleus and an increase in the nystagmus. This lasted approximately 4 sec., after which time both the nystagmus and the IFSP became more regular in form.

Stimulation of the superior colliculus required slightly higher parameters to elicit a change in the nystagmic and cerebellar response In *S* 412 (Fig. 16) there was inhibition of both potentials during the period of stimula tion with stimulus offset, there was an increase in th nystagmic response and a return of the IFSP

Stimulation in the Anesthetized S

In an attempt to separate the effects of cortical (Cohen *et al.*, 1965) and sub-cortical electrical stimulation *Ss* were stimulated while under sodium pentobarbital Each *S* was run at only one of five dosage levels ranging from 20 mg/kg–45 mg/kg. The animals were placed in the apparatus within 30 sec. of the injection, making it possible to monitor the immediate effects

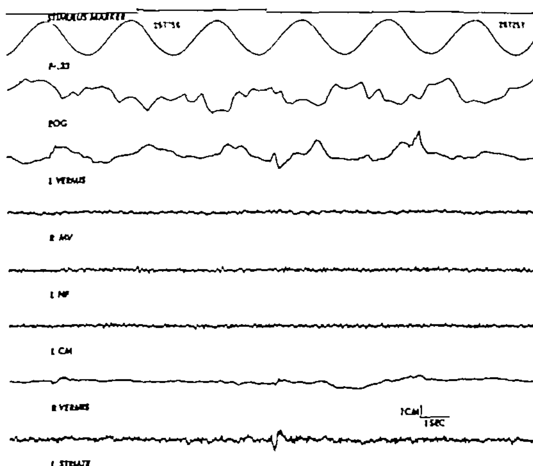


FIG. 14 S 417 Stim lati a f MRF (10 μ mps., 250/sec 1 msec) Stim l tl led t increase i the nystagmic response nd verm l potential. EOG 500 μ /cm, vermal 100 μ /cm, all thers 50 μ cm, 25 mm/sec

of the drug on the nystagmic and EEG responses. Even with the lowest dosage level there was complete suppression of all vestibular nystagmus. This was true for all *Ss* in which anesthetic induction was complete In those *Ss* with good (MRF) electrode implants, a brief stimulus (1–3 sec.) resulted in an almost immediate return of the slow phase of nystagmus *S* 333 was run under the highest level of the drug (45 mg/kg) and as shown in Fig 17 stimulation of the mesencephalic reticular formation led to a return of the slow phase The slow phase was always compensatory and as long as the animal was being oscillated, showed no decrement in amplitude If the oscillation were discontinued for a period of 5 min. or more and then reinitiated, there was again complete absence of nystagmus until the MRF was stimulated electrically With shorter inter trial intervals the slow phase f nystagmus would reappear with stimulus onset In *S* 413 (Fig 1) the MRF was stimulated before nystagmus was completely suppressed This caused an immediate increase in the potential from the left

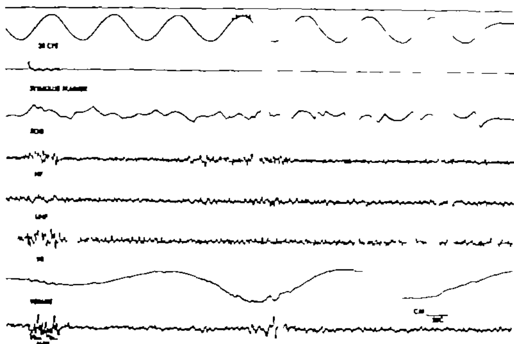


FIG. 15. S 328. Stimulation of MRF during habituation. Stim 1 onset 250 μ mps., 1 msec., 250 sec.) led to little or no change in EOG or vermal response. Five and one-half sec. after stimulus offset, IPSP increased following spontaneous discharge of RNF. Nystagmic response also showed subsequent increase in intensity. EOG 500 μ cm, vermis 100 μ cm, all others 50 μ cm, 25 mm sec

vermis. When this potential reached its maximum, there was a correlated change in the EOG. Stimulus offset led to a reversal in polarity of the cerebellar potentials and a shift in eye position to the opposite side.

As in the unanesthetized situation, stimulation of the superior colliculus (SC) led to changes in the cerebellar and nystagmic response. One must bear in mind the possibility of responses being due to current spread to the MRF or brachium conjunctivum. During the period of stimulation, there were no apparent changes in the EEG, except for some suppression of the striate cortical response. With stimulus offset, there was a large amplitude increase from the left *vermis* and a corresponding (although lower voltage) change in the EOG.

The most clear-cut and consistent results were obtained with electrical stimulation of the fastigial nuclei. Many unsuccessful exploratory trials were conducted until it was discovered that rapid eye movements could be readily elicited with very brief high frequency (250/sec.) stimulation of these nuclei. Stimuli as low as 20 microamps were effective and as the stimulus was increased in intensity there was an obvious gradation in the magnitude of the response. Stimulation of the right fastigial nucleus in S 409 led to an immediate rapid eye movement to the left. With a current

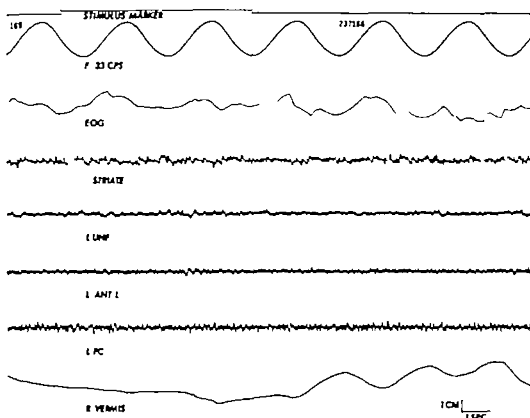


FIG. 16 S 412. Stim l tion of SC in the una esthetized S (60 μamps, 1 msec 100/sec) Stim lation led t l hibition f the nyst gmic response d the ermal potential. With stimulus ffect there wa n incre se i both f these respo ses. EOG 500 μv/cm, erml 100 cm, ll thers 50 μ /cm, 23 mm sec

increase of only 10 microamps there was a slight but noticeable increase in the velocity and amplitude of the response (Fig 18) The record from S 413 (Fig 18) shows the effect of higher current stimulation. There is some variability in the amplitude of the FOG and this is probably due to the repetitive stimulation and the fact that the stimulus was applied at different points of the oscillation cycle

The artifacts seen on some of the channels during stimulation are caused by poor stimulus isolation This is characteristic of the constant current stimulat r when low current values are used However the record from S 413 (Fig 18) shows clear evoked potentials in centrum medianum in response t the fastigial stimulation

None of the S wa stimulated in centrum medianum while under Nembutal Alth ugh two S were timulated in what was believed to be centrum medianum hi t logical tudy h wed the electrodes were actually in the mesenceph ic reticular f rmati n Stimulation of the anterior lobe of the

tet olo-l rgny S ppl 231

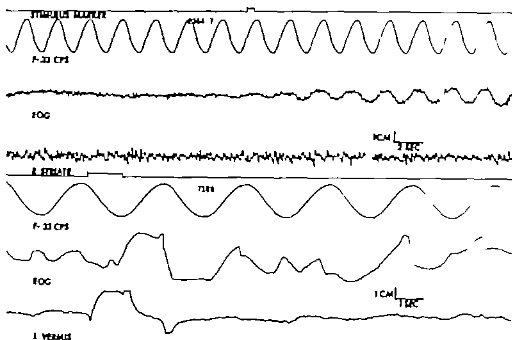


FIG. 17 Stimulation of MRF in anesthetized Ss 333 and 413. In the top record, brief stimulus (75 sec, 200 μamps, .1 msec 300 sec) led to a return of the slow phase of nystagmus for S 333. In the bottom record S 413 was stimulated before complete anesthetic induction had taken place. Note that the vermal response precedes the EOG change. This record also shows that the vermal potentials are not eye movement artifacts, since subsequent wandering eye movements are not reflected on the record from the vermis. Top record EOG 200 μ/cm, striate 50 μ/cm, 10 mm/sec. bottom trace—EOG 500 μ/cm, vermis 50 μ/cm, 25 mm/sec

cerebellar vermis, dorsal uncinate fasciculus, and the nodulus did not cause changes in the EOG. A complete summary of the stimulation sites and results are given in Table 2.

Effects of Cerebellar Lesions on Habituation

It was predicted that partial lesions of the tuber vermis should lead to marked changes in the habituation of vestibular nystagmus, for the following reasons:

1) The cerebellar infraslow potentials decreased in amplitude as *S* habituated.

2) The correlation between the faster vermal potentials and the EOG tended to indicate some primary vestibular control function for this structure. This was further supported by the correlated loss of the fast phase of nystagmus and the cerebellar potentials while under Nembutal.

Following surgery *S* 328 displayed marked hypermetria of the right hindleg, which is typical of a lesion of the tuber vermis. Other than this, the animal was in good physical condition and began eating the day after

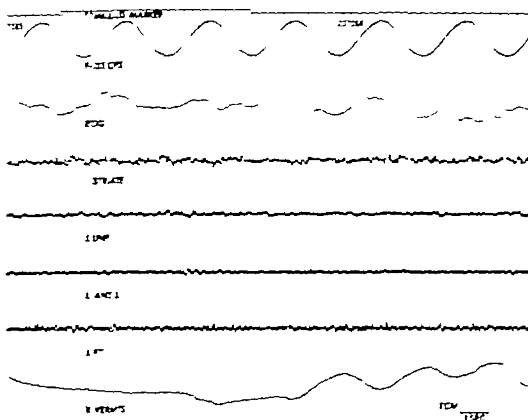


FIG. 16. S 412. Stimulation of SC in the unanesthetized S (.6 mamps, .1 msec 100 sec. Stimulation led t[o] [in]hibition of the nystagmic response and the [...]ernal potential. With stimulus offset there was an increase in both of these responses. EOG 200 µv cm. [...]emus 100 [...] cm. all others 50 µv cm, 25 mm sec.

increase of only 10 microamps there was a slight, but noticeable increase in the [v]el[oc]ity and amplitude of the response (Fig. 18). The record from S 413 (Fig. 18) shows the effect of higher current stimulation. There is some variability in the amplitude of the EOG and this is probably due to the repetitive stimulation and the fact that the stimulus was applied at different points of the oscillation cycle.

The artifacts seen in some of the channels during stimulation are caused by [...] stimulus [...]dation. This is characteristic of the constant current stimulator when low current values are used. However, the record from S 413 (Fig. 18) shows clear evoked potentials in centrum medianum in response to the [...] stimulation.

[...] the S was stimulated in centrum medianum while under Nembutal. Although two S were stimulated in what was believed to be centrum medianum, histological study showed the electrodes were actually in the mesencephalic reticular formation. Stimulation of the anterior lobe of the

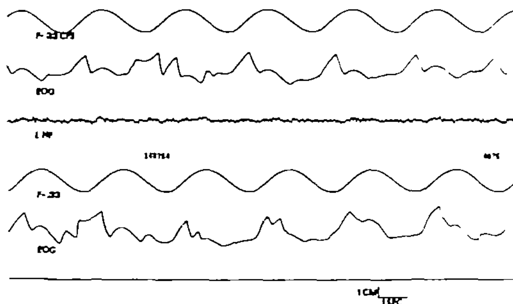


FIG. 20. Ss 323 and 1288. Top record tak n fter 21 trial (1 trial day how ng ı-directional habituati n. Bott m record from S 1358 taken after eight day f timulati n (1 trial/day) Not lmost compl t bsence f the response t the left toward the end of the record. EOG 500 μv m, L \F 50 cm. 25 mm sec



(A) R trectie f l le n S 328



(B) R onstructie f lesion n S 1388

FIG. 21 Reconstructi f lesions (A lesi showed slight in asion of pyramis VII B was complet ly rem d B no l ement with pyramis or parafloccul ; VII B was complet ly removed.

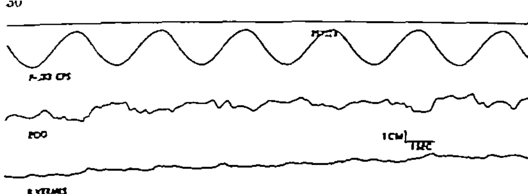


Fig. 22. Habituated record from S 428 taken on th first trial. Not the distortion f f t phases t the left, and flattening of the compensatory movement on the fifth cy l t the left. EOG 500 μv cm. B erml 100 μ cm. 25 mm sec

surgery Fig 19 shows a continuous strip of record taken 32 hours post operatively At the beginning of the oscillation, S displayed a normal nystagmus, with the exception that there was approximately a 30% increase in the overall amplitude of the nystagmus as compared to the preoperative level The most dramatic change took place approximately 24 sec. after stimulus onset, at which time S habituated to rotation to the left while maintaining nystagmus in response to rotation to the right To further confirm this finding, the same operation was performed on another animal Because S 1388 was more debilitated by the surgery it was not run until 48 hours postoperatively However an almost identical response pattern was obtained. The animal had a normal nystagmus for the first 30 sec and then habituated unidirectionally to rotation to the left.

On the second day (of and 72 hours postoperatively) both Ss had a "normal" vestibular response to oscillation S 328 did not habituate in either direction, and S 1388 showed only a brief (5 sec.) habituation t rotati n to the left. Subsequently both Ss were run for varying periods (2–8 mins. every day until bilateral habituation took place S 328 showed little or no ign of habituati n for a period of 18 days. At this time there was a return to the pattern seen 32 hours postoperatively (Fig. 20) The S howed habituati n when accelerated to the left and an "overcompensa tion" to the right It was not until the twenty first day of stimulation that the animal h wed habituati n in both directi ns In the case of S 1388 there wa c mplete breakdown in the vestibular response to rotation t the left after eight days of timulation (Fig. 20) Again, the animal reflected an over-c mpensati n in the opposite direction. On the twelfth day of stimulati n, thi S als h wed habituation in both directi ns. Fig 21 h ws hi t l gical rec n tructions f the lesions in these two Ss.

Hi tolo cal tudy revealed that S 428 had a lesion f the rostralmedial p rtion f th left fa tigial nu leus, and some tissue destruction in the right fa ti ial A m nti ned, thi animal did not have a normal nystagmus, and the rec rd fr m th first trial indicates marked habituation (Fig 22 There are v ry few fa t phases t th l ft, and those that can be identified are of low am plitud In ddition the compensat ry movement is irregular

DISCUSSION

Slow Potentials

One of the most dramatic findings in the present experiments was the slow potentials recorded from the cerebellar tuber vermis. As early as 1890 Beck had been able to record slow potential changes from both cere bellar and cerebral cortex. More recently Arduini (1961) found that slow potentials could be recorded from the cerebellar cortex in response to sensory nerve or midbrain reticular stimulation Prior to this, Dondey & Snider (1955) found that electrical stimulation of the cerebellum led to changes in slow D C. potentials recorded from the cerebral cortex. Although Russian investigators have done extensive research on cerebral slow potentials (Aladjalova, 1964) little is known of the functional significance of cerebellar slow potentials.

Typically slow potentials are recorded with D C. amplification since this permits greater amplitude and lower frequency recording However one is always faced with the problems of electrode polarization and amplifier drift Before turning to a discussion of slow potentials and their relevance to vestibulo-cerebellar function, it is best to consider artifacts which could give rise to such potentials.

1) The IFSPs were not due to respiration, since they decreased in am plitude with time and had a very low frequency (5–7/min)

2) They were probably not the result of varying blood flow This change in blood supply would have to be very discrete since the potentials varied over a few millimeters of recording area.

3) A.C. amplification was used in the present experiments and therefore any D C. shifts in potential would be blocked by the capacitance coupling Furthermore *Ss* were not run until 10 days postoperatively leading to a polarized silver-silver chloride electrode

4) The most likely consideration is that the potentials were due to volume conduction from the electrical field of the eyes However as the records indicate the IFSP was often of a higher amplitude than the eye-movement potential and was usually not in phase with the eye movement potentials. The fact that the cerebellar potentials often led the eye movements by one-half second also argues against their being artifacts due to volume conduction from the eyes

The physical basis of slow potentials is, in itself a difficult problem to assess It has been proposed that they are due to large changes in meta bolic rate in response to sensory stimulation (Aladjalova, 1964) These changes are then reflected as impedance variations at the cortex. The present discussion, howe er will be limited to the possible significance of these potentials in vestibulo-cerebellar function.

Overlap of Sensory Function at the Cerebellum

The thesis that the cerebellum exerts a major control over vestibular function is not new for example Flourens work in the early 19th century Halstead (1935) was the first investigator to propose that habituation of vestibular nystagmus in the pigeon was under cerebellar control Unfortunately he did not report adequate histological data. His proposal was, however confirmed indirectly by DiGiorgio & Pestellini in 1948 when they found that guinea pigs with lesions of the tuber vermis did not show habituation to a rotatory stimulus. The fact that the present results are similar for carnivores further supports these data

Slow potentials appeared in all animals prior to stimulus onset, thus indicating that they were related to other sensory inputs. The tuber vermis of the cerebellum receives sensory information from the spinal system via the spino-cerebellar tract. Input from the labyrinth is by way of projections from the Purkinje cells of the flocculonodular lobe and fibers which pass directly from the labyrinth to the fastigial nuclei as the vestibulo-cerebellar fasciculus (Ranson, 1959) Snider & Stowell (1942 1944) Snider & Eldred (1952) Deura & Snider (1964) have shown the overlap of visual auditory and somatosensory inputs on the cerebellar tuber vermis These results were supported (in the cat) by Munson s (1965) establishment of a direct tecto-cerebellar tract This polysensory overlap is relevant to the data obtained in the present experiments.

Much of the research on the vestibular system has been hampered by the assumption that nystagmus and other eye-movement responses are the result of a specific neural structure For example research on thresholds of the semicircular canals is often interpreted as though these were the only sensory structures being stimulated. Dodge (1923 *a*) was one of the early investigators to emphasize the problems of proprioceptive auditory and visual inputs confounding threshold determinations. Although some investigators have paid token attention to isolated factors, yet they have ignored the potential interactions of these sensory systems. This same research attitude has prevailed in some cases with "pure" cerebellar research Resp nses to electrical stimulation of the cerebellum have been interpreted independent of the possible interaction of the vestibular apparatus. Though evidence now exists (Carpenter & Strominger 1964) for direct connections from the cerebellum to the oculomotor nuclei the major influence on the oculomotor system does appear to be mediated by the vestibular nuclei

It can be argued that the vermal slow potentials recorded in the present experiments reflect an integrated measure of the tonic and phasic activit of the [illegible] lar proprioceptive and kinesthetic systems of the organi m The larg [illegible] sinusoidal wave activity seen in the early trials may in part [illegible] the degree of immobility which the animals di played The fa [illegible] in response to the vestibular stimulation reflect pha ic reacti n [illegible] ted t the ny tagmic response Although no direct

[illegible]

quantitative measures were made th ... ity of these potentials were indicative of the result ... quency and amplitude Since almost all of the records ... taken from the second turn of the tuber vermis, the f ... primarily related to fast phase eye movements to the le ... ment with Cohen *et al* (1965) who have shown that ele ... n of this portion of the vermis leads to conjugate horizont ... movements to the left Stimulation of the more medial porti ... ber vermis leads to rapid conjugate deviations to the right

Cohen *et al* (1965) also believe th ... a three dimensional topographic representation for eye movem ... t both the cortical and subcortical level of the cerebellum Stim ... at different cortical points leads to eye movements in three planes ... closely approximate those eye movements elicited by electrical stimulation of the semicircular canals This representation is also maintained at the level of the roof nuclei

Relationships of the Tuber Vermis and Fastigial Nuclei to Nystagmus

The projections from the vermal cortex to the fastigial nuclei are further supported by the electrophysiological results of the present experiments Increased firing and alternations in voltage of the fastigial nuclei and uncinate fasciculus were associated with the peak of the vermal potentials It is especially interesting that these were correlated with right vestibular nuclei and initiation of a fast phase to the right The fact that those Ss with lesions displayed a type of "over-compensation" to the right is also in agreement with the theory of algebraic summation by the vermis

Fernández and Fredrickson (1964 p 61) found that stimulation ... nodulus in the cat led to partial inhibition of nystagmus, and ablati ... this structure resulted in prolonged vestibular reaction to stimu ... These investigators postulated the nodulus acting as an inhibit ... sory input to the vestibular nuclei and the reticular formation ... the changes in the nystagmu during stimulation, they also con ... impulses from the nodulus were affecting the neural mechani ... fast phase An extremely relevant consideration is that th ... addition to sending efferent fibers to the vestibular nuclei ... formation, also sends inhibitory fibers to th fastigial nucl ... ruzzi, 1958) It is interesting to note that the one S (428) ... experiment with a partial lesion of the left fastigial nucl ... some slight involvement of the right side) showed an ... from the beginning This further supports the hypothesis th ... nuclei are the final control mechanism for the fast ... nystagmus.

The two Ss with vermal lesions also showed an occur ... the compensatory phase of nystagmus to the left. In ... pensatory movement became distorted and in phase ... This type of response was never seen in over 100 tri...

(Wendt (1965) has postulated that distortions of the slow phase which are often seen during habituation in primates, are due to an active intrusion of the fast phase mechanism upon the compensatory pattern. The data from the present experiments can be interpreted to support this theory

Role of the Reticular Formation in Habituation of Nystagmus

One of the major problems in understanding habituation of vestibular nystagmus has been the necessity for invoking the reticular formation as the probable control center of the fast phase of nystagmus. There is a great deal of literature in support of this theory but it has been based on highly tenuous premises which may not be valid. There is little doubt that habituation is an active central process. Thompson & Shaw (1965) have established that for the auditory system there is no fatigue at the sensory end organ or relay nuclei in response to 74 hours of continuous stimulation Therefore any modifications in the response must take place "more centrally" This also appears to be true for the vestibular apparatus. In the present experiment, there was no decrement in the compensatory phase of nystagmus after three hours of continuous stimulation (animals under Nembutal and one S under chloralose) The fact that animals under deep barbiturates and ether anesthesia display no fast phases has been used as one argument for reticular formation control (Szentágothai, 1943 Hood & Pfaltz, 1954) In the present experiments, the potentials from the cerebellar tuber vermis also disappeared under Nembutal Stimulation of the reticular formation in the unanesthetized S led to an increase in the fast phase frequency (i.e., loss of habituation) of the nystagmic response but also caused a simultaneous increase in the potentials from the cerebellum However reticular stimulation under deep Nembutal never elicited a rapid eye movement but only a return of the slow phase of nystagmus. Low current stimulation of the fastigial nuclei, under the same conditions, always elicited a contralateral fast phase In the lightly anesthetized S MRF electrical stimulation led to a sharp increase in the vermal potentials which preceded the EOG change by as much as one half second

Another argument for reticular control of nystagmus has been that completely decorticated (neocortex) cats show normal habituation to a rotatory stimulu (Fernández & Schmidt 1963 Hernandez-Peón & Brust Carmona, 1961) Fernández & Schmidt (p. 16) conclude " the phenomenon apparently takes place in lower centers, probably in the vestibular nuclei the reticular formation of both medulla and pons or in both these centers" What is important in their discussion of the problem is the qualification which they subsequently make "These structures, however seem to be und r a tr ng cerebellar influence because localized or extensive cerebellar l si n (1 16) r ablati n of the nodulus (45) prevent acquisition unt [illegible] l c mpensates for cerebellar deficiency" (The relation hip [illegible] n d ilu nd the fa tigial nuclei has already been discu sed [illegible] the finding f Halstead and of DiGiorgio &

[illegible]

Pestellini) As the authors imply the findings of habituation in decorticate cats does not provide a conclusive argument for reticular control, since the cerebellum was 100% intact in these Ss The present data also bring into question the conclusion that an animal does not habituate until it compensates for cerebellar deficiency Ss 328 and 1388 could not have been more deficient than they were 32 and 48 hours postoperatively yet both showed rapid unidirectional habituation Secondly these Ss never compensated for the deficiency based on their nystagmic or behavioral responses. Both showed a preponderance of nystagmus to the right, and over stepped until sacrificed 30 and 26 days postoperatively

A third, and probably the most valid, argument for the importance of the reticular formation in habituation of vestibular nystagmus is that the state of alertness appears to be related to the process of habituation. Manipulation of arousal such as by drugs or in the case of humans, by mental tasks, has an effect on the process of habituation Amphetamines are believed to act on the reticular formation, but in the high doses commonly used in vestibular studies with animals they sometimes cause ataxia which may be cerebellar in origin The question arises concerning the effects of this drug on potentials at the cerebellar cortex. The fact that dishabituation took place in the present experiments in response to auditory and visual stimuli lends support to the reticular hypothesis, but is again confounded by the associated changes which take place at the cerebellum The fastigial nuclei and reticular formation reciprocally innervate each other thereby making it difficult to separate cause and effect A useful experiment which would aid in answering this question would be to ablate or stimulate the tuber vermis to see if there are changes in the degree of habituation to a stimulus of some modality other than vestibular

Vestibulo-Cerebellar Interactions with Sensory Stimuli

If the cerebellum is a major link in the control of vestibular nystagmus, then one should be able to logically explain vestibular interactions with other sensory modalities. The tuber vermis responds to auditory visual and somatosensory input (Deura & Snider 1964) and, as these authors point out, other investigations have obtained data which support this convergence of sensory input Clark & Graybiel (1948 p 6) found that a S's ability to localize a sound coming from a source directly in front of him was impaired if S were rotated and then brought to an abrupt stop The sound was displaced, an average of 17 in the direction of the slow phase This displacement gradually decreased to 0 25 to 30 sec. later This period is approximately how long it would take for the sensation of rotation to run its course These authors conclude " this constant error in sound localization, or what might be termed the *audiogyral illusion* is based upon the complex intera tion of two sensory systems and the resultant perception is the S's re t n to this total situation which produces gross constant errors in the l t on of sounds.

RÉSUMÉ

Les enregistrés faits électrophysiologiques de la structure cerebellovestibulaire dans non-anaesthetisés et anaesthetises chats indiquent que le cerebellum folium tuber vermis et noyaux fastigiaux jouent un primaire rôle dans le controle de la phase ferme nystagmique et dans le procès de la habituation vestibulaire Quinze chats ont été implantés avec les électrodes chroniques dans la structure cerebellovestibulaire et répété exposés à la oscillation horizontale Les infralents potentiaux enregistrés de cortex cerebelleux se diminuaient en amplitude quand les sujets se habituaient a la stimulation vestibulaire La stimulation électrique de la formation mesencephalique reticulaire sous Nembutal conduisait au retourner de la phase lente de nystagmus (les animaux ont eté oscillés) pendant que la stimulation électrique de noyau fastigial sous les mêmes conditions toujours portait dehors la phase ferme contralatérale Une blessure discrète de tuber vermis conduisait à rapide habituation unidirectionelle à la stimulation vestibulaire Ces faits (évidences) supportent la hypothèse de Brodal (1960) que le noyau fastigial exerce le controle sur les noyaux vestibulairs et en resultant les réponses oculomotores. De plus, le folium tuber vermis parait d'être la structure primaire controllant la habituation par son action directe inhibitrice sur le noyau fastigial

SUMMARY

Electrophysiological recordings from cerebello-vestibular structures in unanesthetized and anesthetized cats indicates the cerebellar folium tuber vermis and fastigial nuclei play a primary role in control of the nystagmic fast phase and the process of vestibular habituation. Fifteen normal cats were implanted with chronic electrodes in cerebello-vestibular structures and repeatedly exposed to horizontal oscillation. Infraslow potentials recorded from cerebellar cortex decreased in amplitude as the subjects habituated to the vestibular stimulation. Electrical stimulation of the mesencephalic reticular formation under Nembutal, led to a return of the slow phase of nystagmus (animal being oscillated) whereas electrical stimulation of a fastigial nucleus under the same conditions always elicited a contralateral fast phase. A discrete lesion of the tuber vermis led to rapid unidirectional habituation to vestibular stimulation. These data support Brodal's (1960) hypothesis that the fastigial nuclei exert control over the vestibular nuclei and resulting oculomotor responses. Furthermore the folium-tuber vermis appears to be the primary structure controlling habituation through its direct inhibitory action on the fastigial nuclei.

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APPENDIX

Instructions for reading EOG records

Note In all records, the decreasing cosine wave (90–270 degrees) indicates acceleration to the left while the increasing cosine wave (270–90 degrees) indicates acceleration to the right This can also be interpreted as movements up being equal to acceleration to the right and deflection downward acceleration to the left

In the case of the EOG there is an exact 180 degree reversal. Upward movement indicates an eye movement to the left, and a downward deflection an eye movement to the right. The eyes typically reverse direction around the points of zero acceleration (maximum velocity) at 0 and 180 degrees. Due to the low velocity of the cats saccadic eye movements, there is often difficulty in determining a "true" fast phase

List of Abbreviations

AE	A teri Ectosylvian Gyrus	SC	Superior Colliculus
CM	Nucleus Centrum Medianum	SN	Substantia Nigra
DH	Dorsal Hippocampu	TCR	Thalamocortical Radiati ns
FC	Fornix Commissure	UNF	Uncinat Fasciculus
HC	Hippocampal Commissure	VH	Ventral Hippocampus
MMB	Mammillary Body	VL	Nucleus Ventralis Lateralis
MRF	Mese cephali Reti lar Formatio	VR	Visual R diations
MV	Medial Vestibular N leus	WMP	Whit Matter Parahippocampus
NOD	Nodulus		

TABLE 1 *Effects of Electrical Stimulation in the Un-anesthetized Animal*

Subject	Histological Locus	Parameters	Results
328	MRF	250 microamps, 1 msec 300/sec.	5 seconds post-stimulation, increase in IFSP and nystagmus
333	MRF	200 microamps, 1 msec 300/sec	Immediate increase i fast phase frequency and decrease in amplit de of the nystagmus
409	MRF	30 microamps, 1 msec 250/sec	Immediate increase in fast phase frequency and decrease in amplitude of the nystagmus, disruptio of IFSP
415	MRF	75 microamps, 1 msec 300/sec.	Immediat increase in fast phase frequency and decrease in amplit de of the nystagmus, disruption f IPSI
427	MRF	10 microamps, 1 msec 100/sec	Increase in overall frequency nd am plit de f the nyst gmus, increase i frequency of the IFSP with stim lus offset
412	SC	50 microamps, 1 msec	Inhibition f nyst gmus nd IFSP with stimulus offset increase in nyst gmus and IFSP
402	SC	150 microamps, 1 msec	Increase in frequency of nystagm an I IFSP
391	PH	70 microamps, 1 msec 100/sec	Increase in amplitud and frequency f y tagmus, preceded by hange in IFSP

TABLE 2 *Effects of Electrical Stimulation in the Anesthetized Animal*

Subject	Histological Locus	Parameters	Drug	Result
328	L MRF	100 microamps. 1 msec. 300/sec.	30 mg/kg	Almost immediate return of slow phase of nystagmus
333	L MRF	200 microamps. 1 msec. 300/sec.	45 mg/kg	Almost immediate return of slow phase of nystagmus
409	L MRF	40 microamps. 1 msec. 250/sec.	45 mg/kg	3 second burst of random eye movements
413	R MRF	100 microamps. 1 msec. 250/sec	20 mg/kg	Almost immediate return of slow phase of nystagmus
427	R MRF	100 microamps. 1 msec. 300/sec.	25 mg/kg	Slight increase in amplitude of slow phase of nystagmus
412	L FC	100 microamps. 1 msec. 300/sec.	36 mg/kg	No change in any leads
412	R SC	75 microamps. 1 msec. 300/sec.	36 mg/kg	Increase in slow phase of nystagmus, and return of IFSP
391	L PH	20 microamps. 1 msec. 100/sec	45 mg/kg	Return of IFSP preceding slow eye movement by 1/2 sec.
400	R MRF	50 microamps. 1 msec. 250/sec.	25 mg/kg	Inhibition of slow phase of nystagmus with stimulus offset
400	NOD	25–200 microamps. 1 msec. 300/sec.	25 mg/kg	No change in any leads
400	L NF	20 microamps. 1 msec. 250/sec.	25 mg/kg	Rapid eye movement to right
400	R NF	40 microamps. 1 msec. 250/sec.	25 mg/kg	Rapid eye movement to left
413	R NF	100 microamps. 1 msec. 250/sec	20 mg/kg	Rapid eye movement to left
427	L NT	75 microamps. 1 msec. 250/sec	25 mg/kg	Rapid eye movement to right
412	Ant L	10–100 microamps. 1 msec. 300/sec.	36 mg/kg	No change in any leads
412	L LNF	50–100 microamps 1 msec 10–300/sec.	36 mg/kg	No change in any leads
328	R NF	75 microamps. 1 msec. 250/sec	30 mg/kg	Rapid eye movement to left

TABLE 3 *Sub Cortical Electrode Sites (Histologically Verified)*

328	R NF P 8.5 L 1 5 H 0 0	L UNF P 8.5 L 3.5 H −2.0	L MRF A 2.5 L 1 0 H 0.0	L MRF A 6.0 L 2.5 H 1.0	R VR A 6.0 L 12.0 H 6.0
333	M NF P 8.5 L 0.0	R UNF P 8.5 L 3.0 H −2.0	L MRF electro- physiological	L CM A 8.0–8.5 L 3.0 H 1 0	
398	M NF P 8.5 L 0.0 H 0.0	L MN P 6.0 L 3.0 H −3.5	L MRF A 3.0 L 3.0 H 0.0	M SN A 6.0 L 5 0 H 4.0	
391	M NF P 8.5 L 0.0 H 0 0	Ant Lobe Ctx P 8.5 L 3.0 H 5.0	W MP A 2.0 L 2.5 H 11 0		DH A 6.5 L 3.[illegible] H 8.0
400	L NF P 9.5 L 1 0 H 0.0	Nodulus P 0.5 L 1 0 H −1 0	R MRF A 5.0 L 1.0 H .5	L VH A 0.0 L 11.0 H −2.0	R VR A 0.0 L 12.0 H 7 0
402	L NF P 0 0 L 1 0 H 1.5	R MN P 7.5 L 2.0 H −3.0	L SC A 2.0 L 3 5 H 3 0	R MMB A 8.5 L 0.5 H −6.5	TCR A 7.5 L 13.0 H 7.0
409	R NF P 9 0 L 2.5 H 2.0	L MN P 9 0 L 3 0 H 4 0	L MRF A 3 0 L 4 0 H −.5	R MRF A 5.0 L 3 0 H 1 0	L HC A 3.5 L 11.0 H 7.0
411	Ant Lobe Ctx. P 8 0 L 1.0 H 6.0	L UNF P 8.0 L 2.0 H 3.0	R SC A 3.5 L 3.0 H 1 [illegible]	L FC A 3.5 L 3.0 H 9.0	
413	R NF P 6.5 L 1.0 H 1 0	L MN P 8.0 L 3.0 H −1 0	R MRF A 3.5 L 1 0 H 1 0	L CM A 7 0 L 3.0 H 1 0	R VR A 1.5 L 11 0 H 6.5
427	L NF P 8.5 L 2.0 H 1 0	R MN P 8.0 L 3.0 H 1.0	R MRF A 3.0 L 4.0 H .5	L CM A 7 0 L 2.5 H .5	
428	L NI P 8 0 L 1.5 H 1 [illegible]	Nod lus P 9 0 L 1.5 H 2 0	L MRF A 2.0 L 4.0 H 1 [illegible]	R CM A 7.0 L 1.0 H 1 0	
NYZ	M L NF P 8.5 L 0 0 H 3 0	Nod & L NF P 8 L 1 0 H 0 [illegible]	R CM A 6.[illegible] L 5 0 H [illegible].0	R VL A 10 0 L 4.5 H 1.5	

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An audiometric, genetic and morphologic study in 53 dogs

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SUPPLEMENTUM 232

From the Departments of Otolaryngology and Audiology Karolinska sjukhuset, the department of animal genetics, nutrition and hygien the Royal Veterinary College and King Gustaf V Research Institute, Stockholm, Sweden

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CONTENTS

INTRODUCTION

For the study of many problems on the mode of inheritance and pathogenesis of genetic hearing defects it is necessary to rely on animal experiments. In man the slow reproduction governed as it is by various uncontrollable social factors render incidence analyses hazardous to this difficulty must be added the well known insurmountable one of obtaining specimens in good enough condition to permit histologic studies. The indirect approach via laboratory animals is therefore inescapable It would seem reasonable to suppose that certain types of hearing degeneration in man have exact counterparts in the usual laboratory animals.

It is well established that genetic deafness occurs in many animals, including the mouse, guinea pig cat and dog (Altmann, 1950) In the mouse and guinea pig deafness is often combined with locomotive disturbances of various types and grades. The deaf mouse displays no less than 11 variants of such disorders in genetic deafness in the guinea pig they are manifested as waltzing In the cat and dog deafness is often combined with pigmentation anomalies deaf cats are often white with blue eyes (Bamber 1933) and deafness in the dog not infrequently occurs in races with a white or merle colour including the collie Shetland sheepdog, old English sheepdog, Norwegian dunker hound, great Dane, dachshund, foxhound and bull terrier Hereditary deafness occurs also in races without pigmentation anomalies, including the Dalmatian, poodle dachshund and Rottweiler The Dalmatian is not a merle dog, for though the patches vary in size and number the colour is always a uniform dark shade in contrast to the piebold, dirty looking anomalous pigmentation of the merle dog. Deafness in the dog and cat is rarely if ever combined with locomotive disturbances.

Genetically deaf animals have rarely been subjected to careful hearing studies to ascertain the type and degree of the hearing impairment Observations of the hearing capacity of the mouse suggest, however that in many kinds the faculty of hearing is lost, partly or completely relatively early in life Confirmation has been obtained in electrophysiologic and light and electron microscopic studies, especially on the so-called shaker I mouse

It has been shown in a light microscopic study by Grüneberg *et al.* (1940) and in a light and electron microscopic study by Kikuchi & Hilding (1965) that in the normal mouse the cochlea and the hair cells are not fully developed until 12 days after birth In the shaker I mouse, however degenerative changes could be observed by electron microscopy from the 12th day after which they progressed rapidly

Using the Preyer reflex as a rough test of the hearing Mikaelian & Ruben (1964) found that cochlear potentials could be recorded from normal mice on the eight day but in shaker I mice not until the ninth and never throughout the normal frequency range After 21 days the cochlear potentials could no longer be recorded, nor could the action potentials these did not attain the same magnitude as in the normal animal and disappeared on the twentieth day The Preyer reflex, which normally appeared on the tenth day was not recorded until the twelwth, and disappeared on the twenty second.

The literature contains no evidence of a similar early disappearance of the hearing in cats with genetic hearing defects. Histologic studies by Bosher & Hallpike (1965) show that in the cat with normal hearing the cochlea and hair cells are not fully developed until the twenty-first day of postnatal life In the deaf white cat degeneration of the inner ear begins at only 4–6 days of age and then progresses. Since the degeneration begins so early it is hardly surprising that there are no reports on the hearing in kittens The degeneration is, according to Bosher & Hallpike limited to the *cochlea and saccule and can be unilateral*

Nor in the case of the dog is there any reported evidence of such early deterioration of hearing, or surprisingly enough any histologic study of the time at which the organ of Corti is fully developed.

In a histologic study of an unspecified number of clinically deaf collies and Dalmatians 2–4 months old, Lurie (1948) found no anomalies of the temporal bone or middle ear but there were degeneration of Corti's organ and atrophy of the cochlear nerve, spiral ganglion and partial collapse of the saccule The nerve degeneration however was not as great as might be expected from the cochlear conditions. The remaining parts of the vestibular labyrinth were normal

In two 2 month-old deaf Dalmatian pups with no electrical response to sound, either pure tone or click, Hudson Durham & Ruben (1962) performed light microscopic examinations. The histologic alterations consisted in degeneration of hair cells and collapse of the saccule but there was no change in the spiral ganglion cells, the VIII nerve or segments in the central auditory system, such as previous reports had indicated This was interpreted as indicating that the neural degeneration has still not developed at this early age

To summarize, no systematic audiometric examination or studies of the mode of inheritance seem to have been performed on dogs with genetic hearing impairment The few animals that have been examined have been judged to be clinically deaf and therefore no audiometric study was considered indicated. The studies that have been carried out have been limited to electrophysiologic and histologic observations.

The hereditary hearing defects in both animals and man can be divided into two main groups (I) *pure bone malformations*, which *affect* conductive or neurogenic mechanisms or both, and (II) those defects that occur

as a result of a degenerative process in the fully developed cochlea. This degeneration, the cause of which is obscure, can begin as early as in the foetal stage or at any subsequent time in life

The damage that inflicts the genetically deaf Dalmatian is of the degenerative type It is known from experience, moreover that there is no dominant mode of inheritance In these two aspects the genetic conditions resemble the most common hereditary deafness pattern in man, and the Dalmatian would therefore seem to be highly suitable for parallel studies.

Of the greatest interest are the time and site at which the degenerative process in the cochlea begins. To ascertain the time of onset some form of hearing test is extremely difficult, as it requires measurement of the hearing acuity of the animal in the first weeks, or even days, of life—perhaps even prenatally

The question of where in the cochlea the process begins is of particular interest because current studies on man have shown that a large number of the genetic hearing impairments begin in the middle range of hearing This suggests a pattern of cochlear destruction quite different from the more basal location of defects caused by the usual exogenous agents. It was manifestly of the greatest interest to clarify whether a tendency towards a corresponding degeneration pattern could be found in the dog.

The objects of the study reported in this paper were

(1) To examine the mode of inheritance of deafness in a genetically and biologically well defined group of genetically tainted Dalmatian dogs and

(ii) to compare the audiometric and histologic findings with respect to the initial site and extent of the defect in the organ of Corti

AUDIOMETRIC AND GENETICAL INVESTIGATION

Breeding material

The breeding experiments were started with two 18-month-old dogs (nos. 1 and 2, Fig 1) both discarded for deafness by a breeder and related (coefficient of relationship 0 188 Wright) After producing two litters they were sacrificed and used in the study of the histology and ultra structure of the cochlea. The breeding experiments were extended to two more dogs (nos. 9 and 15) related to nos. 1 and 2 The breeding programme is presented in Fig 1 In addition to the 4 breeders, the experimental group consisted of 12 litters with 49 dogs (23 males, 26 females) All the dogs appeared to be in good health none showed signs of vestibular disorders, middle ear infection or pigmentation anomalies

Hearing tests were performed on all the offspring at the age of 4–6 months, and on the breeders (nos. 1 2, 9 and 15) at 12–18 months. After the tests representative cases were sacrificed for histological studies.

Equipment

To achieve the highest possible accuracy in the hearing tests it is essen tial to keep the animal s head situated at a definite point in the sound field of the loudspeaker For this purpose a stand was constructed in which the dog was firmly shackled by the neck (Fig 2) If required the legs and body could also be strapped to the stand The stimulus tones were presented through a loudspeaker attached to the stand and placed in front of the dog and 40 cm distant from the head

An audiometer was used as a tone generator which provided a clickfree onset and decay of the tone and the necessary attenuator over a range of 100 dB in 5 dB steps. The frequencies of the test tone were 500 1000 2000 3000 4000 and 6000 Hz. To obtain the required sound intensity a power amplifier was connected between audiometer and loudspeaker The acoustic calibration of the arrangement was made with a half inch condenser microphone (Brüel & Kjaer) placed in the presumed centre of the animal s head The maximum obtainable sound pressure level was slightly different for the various test frequencees ranging from 95 to 110 dB SPL.

The electric conditioning shock was applied to the dog's pinnae via two clip electrodes. The shock stimulus consisted of faradic pulses presented at a rate of 100 per second, and continuously adjustable in intensity up to 50 volts. The acoustic conditions of the test room were not satisfactory and a limit was set below which no safe threshold determination could be expected (see under Test method)

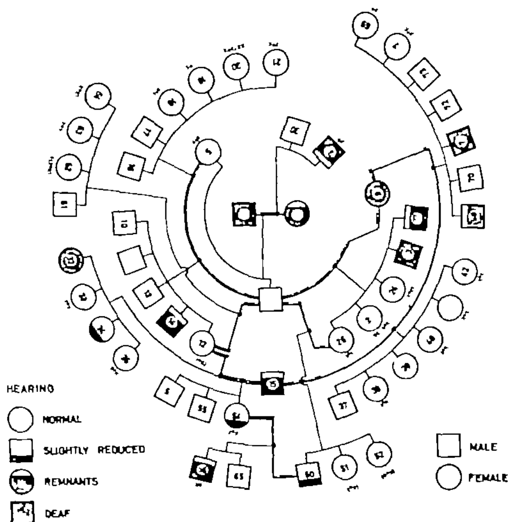


Fig. 1 Pedigree f the 53 dogs sed in the t dy X -chromosome with ormal all l X -chromosome w th defect allele not manifested phenot pically; X -chromosom with defective ll l gl l g rious degrees of deaf ess

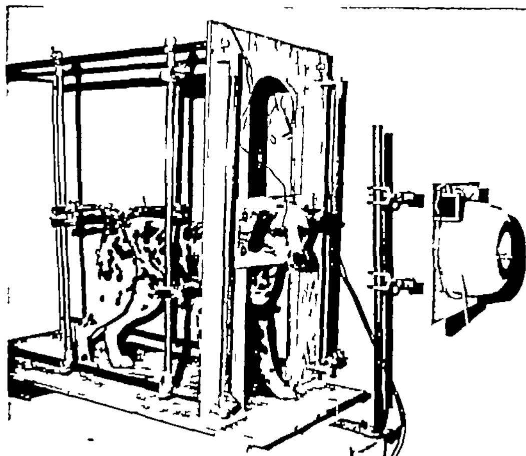


FIG. 2. Hea i g test rra gement

Test method

Attempts were made at first to test the hearing in the puppies when only 2–3 weeks old In these experiments thé lowest level of the test tones required to wake up the puppies was examined but the depth of sleep was found to introduce too great a variation It was therefore necessary to rely upon conditioned audiometry The conditioning procedure was as follows with the dog placed in the stand and the shock electrodes fitted to the auricles, test tones were delivered at random until a level and frequency were found at which the dog seemed to be aware of the sound The avoidance conditioning was carried out by presenting shock-terminated tones at this level until the dog showed signs of anxiety each time the tone was presented The shock was then omitted and the intensity of the test tone decreased in 5 dB steps the threshold for each tested frequency was taken as the lowest tone level at which the dog responded with anxiety whether in the form of whining, barking or general agitation The conditioning procedure usually required 10–20 repetitions and the result showed generalisation with respect to the different test tone frequencies.

This technique proved suitable for dogs from about 4 months of age and the first tests were therefore performed at this age

The acoustic conditions of the test room with respect to reverberation and noise level were unsatisfactory On the bases of noise measurements and hearing tests in dogs of other breeds with clinically normal hearing the lowest safe limit of the threshold test was set at 35 dB SPL, below which no attempts to determine thresholds were made All the animals were tested at least twice at an interval of one week. Several of them required more than two tests before reliable results could be obtained The main difficulty encountered was the tendency for some animals to disturb the test by whining and agitation, and the test conditions in this respect were much improved by the use of a tranquilizer

Dogs nos. 65 66, 68 71 72 and 73 were tested under the influence of Bulbocapnine (de Jong 1945) a drug that induces a cataleptic condition the intention was to exploit this effect to reduce the interference due to general agitation. A dose of 100 mg/kg body weight of Bulbocapnine hydrochloride was administered by intravenous injection

The six dogs tested under the influence of Bulbocapnine reacted somewhat differently during the hearing test The initial reactions to the drug were fairly uniform—salivation and agitation at the end of injection, whining and tremor (in one case aggressive tendencies) after 10–15 minutes and catalepsy after 15–30 minutes. In two cases catalepsy appeared earlier and passed into a somnambulistic condition these dogs were almost apathetic and reacted indistinctly to electric shock and tone the dose was evidently too high. For four of the dogs the drug greatly facilitated the hearing test as the whining barking or general agitation disappeared. In two of the dogs (65 and 66) the tests were impossible to carry out without this treatment.

Results of hearing tests

All 53 dogs were tested by the described method. 38 responded to tones at all the applied frequencies down to a level of 35 dB SPL, and their hearing was thus regarded as clinically normal Five dogs (nos. 1 9 23 32 1) did not react to tones of the maximum intensity—95–110 dB SPL—at frequencies 500–600 c/s. Seven dogs (nos. 2, 14 15 27 31 66 68) had residual hearing and reacted only in the proximity of the maximum intensities and 3 dogs (nos. 34 50 54) responded to tones at 55–70 dB SPL. Typical audiograms are shown in Fig 3.

Genetic interpretation

Among dog breeders deafness is said to be quite common in the Dalmatian A recessive mode of inheritance has been proposed but there would seem to be no scientific confirmation.

It is evident from Fig 1 that the deafness cannot be ascribed to either a dominant or a recessive autosomal gene with complete penetrance The

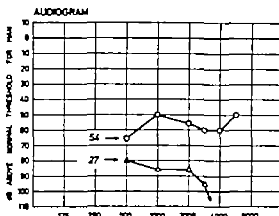


FIG. 3 Typic l dl metric recordi gs (dogs nos. 27 nd 54)

fact the matings between defective parents gave both normal and defective offspring, as did matings between normal parents, is inconsistent with the classical monogenous pattern

The evidence would indicate the action of a sex linked gene Among the total offspring 8 out of 23 (i.e 0.35) of the males and 3 out of 26 of the females (0 12) had hearing loss. The difference is not significant ($\chi^2 = 2\ 0$) Also interesting is the fact that no mating combination gave both deaf males and females

If an X linked gene is assumed the results are consistent with theory only if this gene displays incomplete penetrance in the female heterozygotes In the males, however the penetrance might well be complete The proposed genotypes have been entered in Fig 1 in accordance with this hypothesis. There is one mating that is in some measure inconsistent with the hypothesis It has to be assumed that all female offspring of the mating between nos 15 and 9 must be unmanifested carriers of the defect gene

Since different degrees of hearing loss were observed, the gene must be assumed to display varying expressivity

MORPHOLOGIC EXAMINATION

Light microscopy

The thoracic aortas of 5 dogs (nos. 1 9 14 23 27) with a hearing loss ranging from 90 dB to total deafness were perfused with Susa s Heidenhain fluid. The temporal bones were then removed, decalcified and embedded in celloidin serial sections were cut and stained with haematoxyline and eosin and by Mallory's method

Cochlear duct The lumen of the basal and second coil of all the totally deaf animals (nos 1 9 23) had almost completely disappeared and Reissner s membrane was collapsed and adherent to the stria vascularis and the remnants of the organ of Corti (Fig 4) In the animals with residual hearing (nos. 14 27) Reissner s membrane was collapsed but not adherent to the stria vascularis of the organ of Corti.

Organ of Corti. In most specimens the organ of Corti was completely destroyed in the basal and second coil and only remnants of hair cells were seen The tunnel of Corti was collapsed and the tectorial membrane dislodged from the organ of Corti this membrane has often collapsed and was found to be adherent to the limbus region (Figs. 5–7)

In the apical portions of the cochleae of the animals with residual hearing an apparently normal tunnel of Corti and distinct rows of outer hair cells were found

Stria vascularis The thickness of the stria vascularis and its vascularity were often greatly reduced. In the regions where the Reissner s membrane was attached to the stria this was thin and sclerotic but never completely absent

Spiral ganglion In some of the animals with a hearing loss of more than 90 dB there was a marked reduction in the number of ganglion cells of the second turn and only fibrous connecti e tissue was seen in this region. It is in these animals that the most advanced changes in the organ of Corti were found All the other spiral ganglion cells were normal (Figs 8 and 9)

Vestibular labyrinth In all animals the sensory and ganglion cells of the saccules, utricles, semicircular canals and the ampullas were normal in appearance (Figs. 10 and 11) In some ears there was slight shrinkage of the saccular lumen.

Endolymphatic sac In a few ears there were signs of hyperacti ity of the endolymphatic sac, dense staining of the endolymph, and the lumen of the sac was filled with floating cells and debris.

FIG. 4 Light microscopical survey of tot lly deaf dog howi g degen rati f the cochlea duct I ll coils. Dog n 1 13

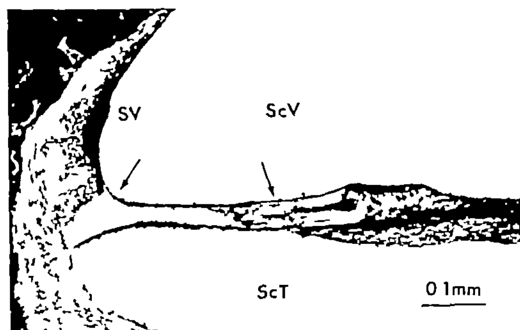


FIG. 5 Apical l f dog l with compl t ly dege erated org f Corti. The stri ascula i i thin nd sclerotic (SV) nd R l an r' m mbra (arrows) i ttached t th stri scul ris d t th collapsed rga f Corti. Sc V Scala vestibuli; Sc T scala tympani 150

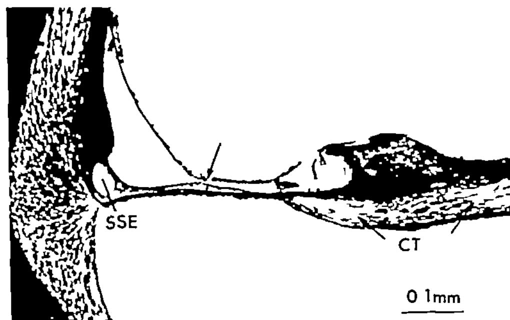


FIG. 6 Second coil f dog 1 with ly ndifferentiated epithelium in the pl ce of the organ of Corti (rrow) Only the l mbus regio with coll psed tectorial membra is still recognizabl The leu pirall xtern cells ha completely disappeared (SSE) Nerv fibres run l g through the haben la perforata can ot be found this coil. Only fibrous connective tissu i present (CT) Compare the same region l the pical coil, Fig 5 d basal coil, Fig. 7 150

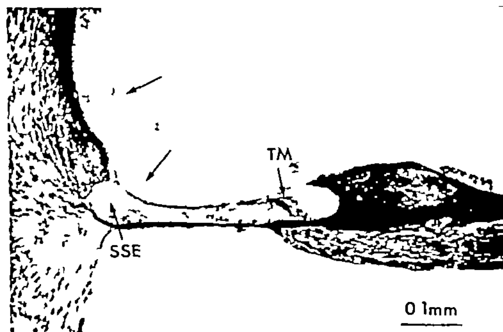


FIG. 7 Basal coil of dog no. 1 displ ying the same changes i the pical coil (Fig 5) t as degeneration of the sulcus piralis ternus (SSE) is found together with coll psed tectori l m mbrane (TM) There is fibrinous tra sudat in the peri l mph tic pace lose t R issner' membrane (rrow) 150

Fig. 8 Detail of ormal piral g nglion cell from the basal coil f dog no. 1 (*SpG*) × 150

Fig. 9 Det il f p ral g glion cell ren f the second coil of dog no. 1 The ganglion cells ha d sappeared nd ly fibrous co necti ti present (*CT*) 150

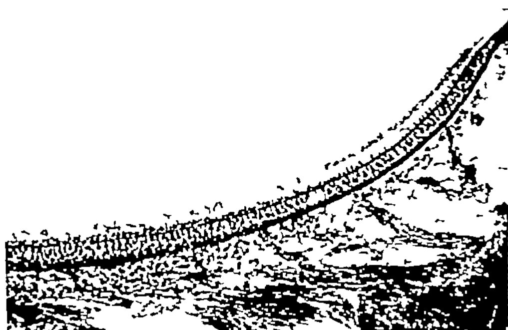


Fig 10 The macula utriculi f dog o. 1 The sensory epitheli m with t lites and the connecti g nerv fibres re normal. 150



Fig 11 The crista mpullari from dog no. 1 hori t l mpulla. The sensory epithelia of ll the mpullae, even those i the tot lly deaf animals, were normal. 150.

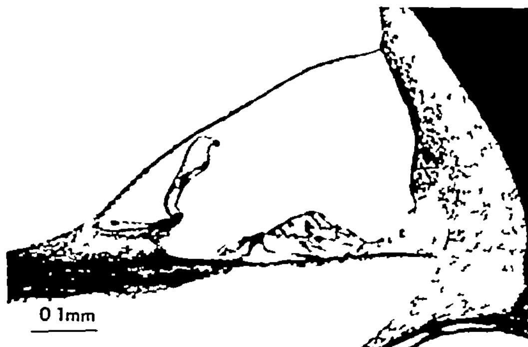


FIG. 12. I dog no. 14 with a hearing los of 90 dB the organ of Corti of the left cochlea wa complet ly degenerated. I the second coil, illustrated here ly rem nt f hai cell re present d th stria scul ri I thin nd sclerotic There is so far n hydrops or shrinkage of the labyri th, a ill trated by the normal position f R issner' membrane 150

FIG. 13. Dog n 14 right side, 2nd coil. This ar ppears t be almost normal (cf. Fig 12) All the hair cell are present d the bundles f sensory hairs can be seen. The stria vasculari and Reissner' membrane re pparently normal. 150

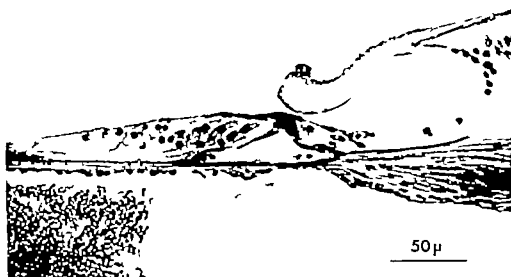


FIG. 14 Dog no. 27 basal coil. Although thi animal recorded ly residual hearing (cf Fig. 3 the org f Corti ppeared t be normal under the light microscope 400.

Slight damage to the labyrinth In two dogs (nos. 14 and 2) one ear showed complete degeneration and the other slight damage (Figs. 12 and 13) The hair cells of the organ of Corti could be recognized in all the turns, although slightly distorted, and there was apparently no degeneration of the stria vascularis or the cochlear spiral ganglion cells (Fig 14) The estibular part of the labyrinth in these ears was normal

Electron microscopy

In four animals (nos. 2, 31 32, 34) with a hearing loss ranging from 35 dB to total deafness the temporal bones were removed under general anesthesia. The middle ear and the round and oval windows were opened and the cochlea was perfused with osmium tetroxide solution through a hole in the apex. The specimens were dehydrated and embedded in Epon epoxy resin by the method of Luft (1961) The labyrinths of these animals were removed with a small rotating saw under an operating microscope the various turns of the cochlea were mounted for electron microscopy and thin sections were cut with an LKB-Ultrotome These were stained with uranyl acetate and lead acetate by Watson (1958) and Karnovsky s (1961) methods. For purposes of orientation 1 μ sections were cut with an Ultrotome stained with toluidine blue and examined under the light microscope

Owing to the extensive degeneration of the organ of Corti of the animals with a hearing loss of 95 dB or more the electron microscopic study of

these ears did not yield more information than that obtained by light microscopy

In one dog (no 34) with a hearing loss of 55–70 dB the changes in the hair cells described below were found One ear was completely degenerated but the other appeared to be almost normal under the light microscope but the electron microscope disclosed severely degenerated hair cells.

Outer hair cells Most of the outer hair cells displayed severe oedematous vacuolar degeneration of the cytoplasm sometimes with rupture of the cell walls (Fig 15) The mitochondria exhibited various stages of degeneration such as swollen membranes, absence of the mitochondrial cristae and sometimes, rupture of their outer membranes The ground substance of the cytoplasm was "vitreous" in appearance and there were few ribosomes (Fig 16) Their nuclei were swollen and irregular in shape and there was a marked reduction of the chromatin content (Fig 17) Intact cuticles with attached sensory hairs were still present in spite of the severe degeneration of the cytoplasm and its organelles.

Inner hair cells The inner hair cells were also degenerated although to a much lesser extent There were an accumulation of the vesicles in the cytoplasm early signs of degeneration of the mitochondria, swollen mitochondrial cristae and changes in volume (Fig 18) The nuclei were rounded and normal in size but the chromatin content was low In these cells the sensory hairs and cuticular portion were apparently normal (Fig 19)

Nerves and nerve endings No efferent nerve endings were found The afferent nerve endings were slightly swollen but most of the mitochondria were normal in appearance (Fig 20) The nerve fibers of the spiral bundle was present in the tunnel of Corti

Stria vascularis and Reissner's membrane The stria vascularis seemed to be poorly developed with sparse capillaries and few cells of the dark type (Fig 21) The protrusions from these cells, however were seen to extend into the capillary region in the deeper part of the stria In some parts of the stria vascularis dense inclusion bodies were found and there was an accumulation of an extremely dense amorphous material in the zone of contact between the stria cells and Reissner's membrane (Fig 22) Often adherent to the stria cells down to the region to the external sulcus cells, this membrane itself appeared not to be impaired (Fig 21)

Supporting structures In the advanced stages of degeneration the cells of Deiters were severely degenerated, with oedematous cytoplasm and swollen, disintegrating mitochondria. Although collapsed, the fibrillar content of the pillar cells was normal

Basilar membrane The basilar membrane was apparently normal but the vas spirale was not demonstrable

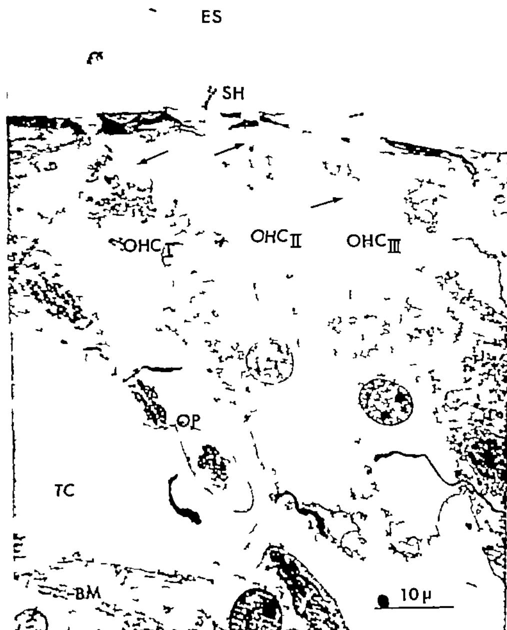
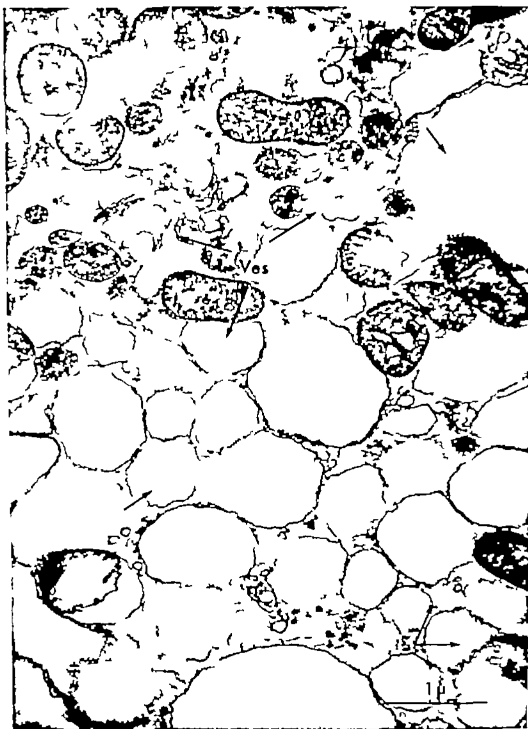


FIG 18. Electro microscopic survey f ter hal -cell region, dog no. 34 Th three row f outer hal cell (*OHC* I III) nd th outer pill cell ca be see (*OP*) *TC*, T nel of Corti *BM* ba il membra *ES* endolymphati pace Note the bea lly calcul ted cyt pl sm f the outer h l cell (rrow) 2000



F o 16 Det il from Fig 15, dog 34 demonstrati g the hea ily esicul ted cytoplasm (Ves) f t hair cell (1st row) lmost dev id f ribosomes d filled with de-g nerating mitoch ndri (rrow) 28,000



Fig. 17 A commo finding i the degenerati g hai cells, dog no. 34 is irregul nuclei (N) with low hromatin content (rrow) Degenerati g mitochondria re also present (M) 23,000



FIG. 18. The inner hai cell IHC dog no. 34 ppr t be almost ormal, lthough the xternal hair cell re degenerated. The nucleus (Nu l not irregular and the hr matin is evenl distributed. The sensory hairs (SH re rmal. Th tun l spiral fibres (rrows nd normal inner pills cell re present (IP T nel f Corti (TC ×3500.



Fig. 19 Det l f Fig 18, dog no. 34 showing the cuticl (C) with sensory hairs (*SH*) of l ne hai cell I this region (Ves) there l slight inciple t calculation of the cyt plasm nd light sw lling f the mitochondria with separatio f the mitochondrial hrista (rrow Dense bodies with irregular "my lin-lik degeneratio patt rn re lso seen (*DB*) 23,000

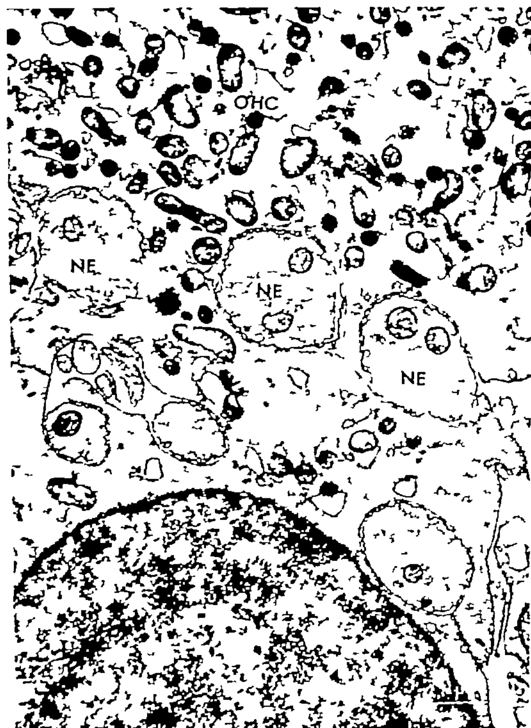


FIG. 20 The basal part f hea fly degenerated outer hair cell (*OHC*) dog o. 34 E en In this advanced t g there are fferent nerv di gs (*NE* with mitoch ndri f lmost normal ppear ce The les f Deiters cell rs n rmal (\x 18,000

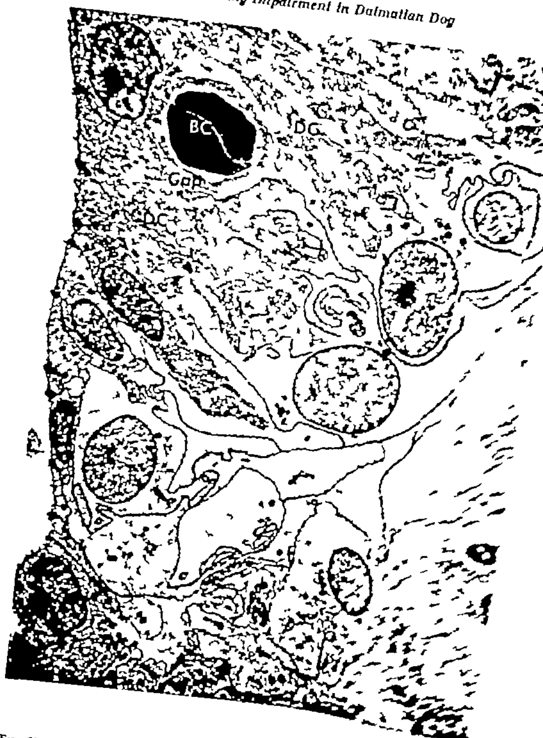


FIG. 21 The tria ascul ri of dog no. 31 i thin and dev loped, ith few dark cell (*DC*) and apparently f lary suppl i sen t but the pill re depleted (*Cap*) o ll lug the l men i occupied by red blood cell (*BC* 4700.

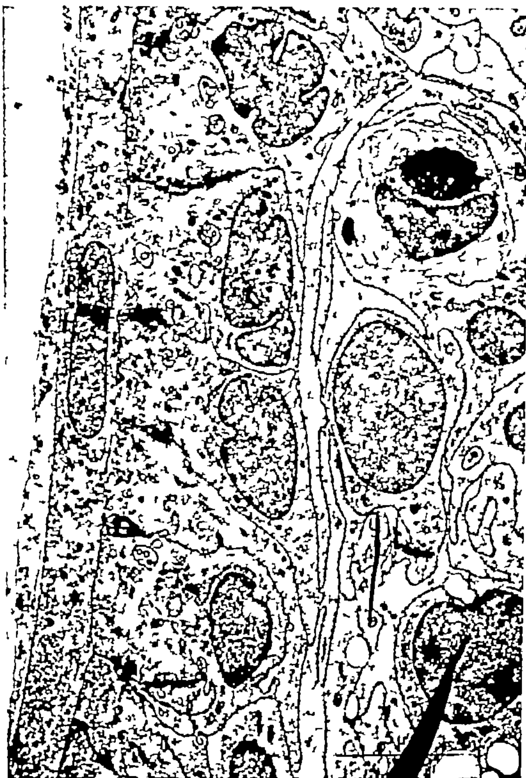


FIG. 22. R lssner's membrane (RM) is coll psed on t the tri ascularis, nd there is dense morphou material t the one f f sion. R lssner' membrane itself l norm l In thi part f the tria scularl there are no dark cells. Dog no 34. 6000

DISCUSSION

To examine the mode of inheritance of the hearing impairments, the dogs were paired in all combinations of affected and normal animals. The results strongly indicate the presence of a sex linked mode of inheritance with a varying penetrance and expressivity If the sex-linked factor is disregarded, there were no obvious differences from man here by far the most common combination, namely children with impaired hearing and normal parents, is represented in the dogs by the pairings 4 × 5 and 4 × 26 (Fig 1) To judge from the different degrees of severity at any particular age it would seem that in the dogs, too there was a variable expressivity of the genes

Attempts to determine the hearing of the dogs within the first weeks of life with various tests yielded no consistent results. Not until 4 months were the animals mature enough for conventional conditioning audiometry to be used This loss of time is regrettable for it makes it impossible to establish whether the hearing organ is fully developed before the onset of the process of degeneration, and therefore prevents a thorough audiometric study of the rapidly progressing early hearing deterioration Future analyses, which will thus have to be conducted on older dogs, will apparently be most time-consuming, since, as in the case of man, the progress in the later stages will presumably be fairly slow and highly sporadic.

In all except two dogs—nos 14 and 27—there were injuries that were visible under the light microscope and that varied in extent with the hearing loss for instance the two totally deaf dogs (nos. 1 and 23) showed a complete degeneration of all the cell elements of the organ of Corti

In the hearing measurements the loudspeaker was placed in front of the head, so that in reality only the hearing of the best ear was measured. The consequence of this procedure is evident from the results for two dogs (nos. 14 and 2) with a hearing loss of 95–110 dB, where the light microscope disclosed total degeneration of the cochlear part of the labyrinth on one side but less severe damage to the organ of Corti on the other an asymmetry reminiscent of the results reported by Bosher & Hallpike on deaf cats (1965) It is possible however that cochleas that appear to be normal under the light microscope might still have cytologic changes. This is illustrated by dog no 34 where a greatly vesiculated cytoplasm, together with disintegrated mitochondria and nuclear changes were found in specimens where light microscopic sections gave no definite indication of degeneration. From the electron microscopic findings—severe degeneration of the outer hair cells but practically normal internal hair cells—it is

evident that the latter cells must have been responsible for the tone perception in this dog

The cytoplasmic changes disclosed by electron microscopy including swollen vacuolar cytoplasm and degenerating mitochondria, and the absence of efferent nerve endings bear a close similarity to those found by Kikuchi & Hilding (1965) in the defective organ of Corti of shaker I mice the nuclear changes in the mice were however less marked than those in the dogs. The vesicular type of degeneration of the cytoplasm and the nuclear changes in some measure resemble the late stage of degeneration in kanamycin intoxication of the organ of Corti (Lundquist & Wersäll 1966) where an observed blockage of the protein synthesis was probably due to degeneration of the ribosomes.

All the earlier investigators have found atrophy of the stria vascularis. In the early stages of degeneration the stria vascularis appeared not to be fully matured, there being poorly developed dark cells and capillary network The cell cytoplasm however was apparently normal, with numerous mitochondria and pinocytotic vesicles close to the endolymphatic surfaces. It is interesting to note that the vas spirale below the basilar membrane was absent

An interesting electron microscopic finding in many animals was that the degeneration of the hair cells in the organ of Corti was complete in the second coil while residues of such cells were found in the apical and basal coils The light microscope disclosed a marked reduction in the number of ganglion cells in the second turn and the presence of fibrous connective tissue in this region. The observations point to general destruction of hair and ganglion cells and stria vascularis in a definite region of the second coil A similar histologic pattern has been demonstrated in guinea pigs with genetic hearing loss—waltzers (Lurie 1941)

The audiometric analysis is complicated by the fact that the normal hearing threshold for the dog is not known for this reason as a reference in figure 3 the human threshold was used, as determined under similar conditions (ISO R 226) As it is unlikely that there are any critical differences within the relevant frequency range, this reference may probably be regarded as tentatively acceptable.

In general the audiometric findings in the dogs is not inconsistent with the location of the centre of degeneration in the organ of Corti, as is indicated by the histological examination Nor is there evidence conflicting with the view that in an early stage this hearing defect may have had the basin shaped appearance so common in hereditary hearing impairment in man. This frequently begins in the range 1000–2000 Hz and then progresses, via a rather flat loss curve into a general high tone loss of an audiometrically fairly unspecific nature

Against the background of these histologic and audiometric observations it is conceivable that this type of genetic hearing impairments has a similar origin in animals and man The typical histologic and audiometric pattern

differs distinctly from that of the usual exogenous damage which is first found audiometrically only in a higher frequency range and where the organic changes are encountered in a more basal part of the cochlea,—the first coil

The explanation of this is probably to be found in the embryonic development of the cochlea which most likely begins in the described region, to extend both apically and basally This course of development finds support in histologic observations on the rat (Lorente de Nó, 1933) and cat (Bosher & Hallpike, 1964) histologic and audiometric studies on the opossum (Larsell *et al* 1935 1944) electrophysiologic studies on the rabbit (Änggård, 1965) and audiometric analysis in man (Johansson, Wedenberg & Westin, 1964) The reason for the degeneration beginning in this area is obscure It may be associated with the fact that it is here that the cells first attain a high degree of differentiation which may be a prerequisite for the initiation of the degenerative process.

SUMMARY

The objects of the study reported in this paper were

(i) To breed a genetically well defined group of hereditary deaf Dalmatian dogs in order to study the mode of inheritance.

(ii) To perform hearing tests and carry out histological investigations of the inner ear and to compare audiometric and histologic findings with respect to the site and extent of the defects

In the resultant group consisting of 53 dogs, five were totally deaf seven had hearing remnants and three moderate impairments, as determined by conditioning audiometry performed from an age of about 4 months. The genetic analysis shows that the deafness cannot be ascribed either to a dominant or a recessive gene with complete penetration It rather indicates the action of a sex linked gene with varying expressivity Apart from this, the mode of inheritance seems to correspond to the most common combination in man i e normal hearing parents–deaf offspring

The audiometric patterns show a flat or gradual loss in the moderate cases a tendency towards a basin shaped curve can be seen

The histologic findings are rather consistent with the audiometric observations. In some cases, where only minor changes could be seen with light microscopy the electron microscopy did disclose severe intracytoplasmatic degeneration of the outer hair cells In the moderate impairments a total degeneration of the hair cells and ganglion cells in the second turn was observed, the destruction, however being much less pronounced in the apical and basal turn of the cochlea

This audiometric and histologic pattern differs distinctly from that of the usual exogenous damage which is first found audiometrically in the highest frequency range and where the organic changes are encountered in the extreme basal part of the first coil The explanation is probably to be found in the embryonic development of the cochlea which most likely begins in the described region, extending both apically and basally The reason for the degeneration beginning in this area may be associated with the fact that it is here that the cells first attain a high degree of differentiation which may be a prerequisite for the initiation of the degenerative process.

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NEURO OTOLOGICAL STUDIES ON BRAIN INJURED EX SERVICEMEN

Follow-up of 256 cases

SEPPO KIRJAVAINEN

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FROM THE OTOLARYNGOLOGICAL HOSPITAL, UNIVERSITY OF HELSINKI (HEAD PROFESSOR URPO SIIRALA, M.D.) AND FROM THE INSTITUTE FOR THE REHABILITATION OF BRAIN INJURED VETERANS (HEAD: PROFESSOR EERO HILLBOM, M.D.) HELSINKI, FINLAND

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Helsinki, February 1968

Seppo Kirjavainen

CONTENTS

I INTRODUCTION

Wartime skull injuries differ in type from peacetime injuries in many ways. Traffic accidents are the commonest causes of skull brain injuries in times of peace. Traumas that originate in this way are usually injuries of concussion-contusion type. Penetrating injuries are less frequent and generally different in character from trauma sustained in war. The incidence of wartime open perforating and penetrating injuries is high. Bullets and shell splinters cause considerable tissue damage owing to their high kinetic energy. In addition, shell splinters because of their shape often have a lacerating effect on tissues. On the other hand, tissue damage caused by bullets in particular may be much more topical than in closed commotion-contusion traumas. Skull traumas which resemble those originating in times of peace naturally occur also during wars.

Quite a number of studies on skull injuries have been published. Detailed audiometric studies have been conducted. The introduction especially of electronystagmography has made it possible to perform more accurate vestibular studies. The majority of the investigations have, however, concerned traumas sustained in peacetime.

Attention was paid back in World War I to the high incidence of hearing defects in war wounded. The literature of that time contains hearing studies and vestibular studies of patients with head wounds. However, the studies are strikingly imprecise. Hearing examinations, for instance, were done by studying speech and whisper hearing. More reliable audiometric studies were performed on the wounded of World War II. Nevertheless, vestibular studies have received less consideration. Therefore a neuro-otological study of persons sustaining brain damage in war seemed to be indicated, especially as good facilities were available for its performance.

II REVIEW OF THE LITERATURE

As the studies on auditory and vestibular lesions caused by war injuries are relatively few in number the review of the literature deals chiefly with hearing and vestibular lesions caused by skull damage in general.

A Hearing Damage

Skull injuries are known to cause auditory lesions. Hearing damage was established in 35 per cent of the patients in the large skull trauma series presented by *Alexander* and *Scholl* (1938) In the material of *Schuknecht* and *Davison* (1956) hearing damage was established in 50 per cent of the patients who had suffered primary loss of consciousness because of the trauma. Impaired hearing as the result of skull trauma was established in 45 per cent of the patients constituting the post traumatic encephalopathy material of *Lumio* and *Aho* (1962) The interval between the trauma and the examination varied in this series from six months to 12 years. *Ey* (1960) reported hearing damage in 44 per cent of his extensive series of 1 000 patients. The examination was made an average of three years after the trauma. At a rough estimate, then, about a half of the persons with skull injuries who are examined suffer from hearing damage. The variations in the different series are readily understandable when it is considered that the nature of the material varies considerably with the different authors. All the materials mentioned consisted of traumas sustained during times of peace.

In thei study of 1954 *Piquet* and *Decroix* stated that a sensori-neural hearing deficiency usually originates as the sequela of skull injury even if there is no fracture of the labyrinthine capsule They also encountered combined forms fairly frequently In contrast a purely conductive hearing impairment was rare. A hearing defect may be manifested as only a slight notch at 4000 Hz. However a skull injury often causes a more marked impairment of hearing over a wider range. Yet, the maximum hearing deficiency is usually established for higher tones. The hearing curve of the pure tone audiogram is thus usually of the descending type (*Schuknecht* and *Davison* 1956 *Proctor et al.* 1956, *Lumio* and *Aho* 1962) *Kecht* (1965) established instances of profound hearing loss in both ears or severe hearing deficiency as the result of even a relatively slight blunt skull trauma. The trauma was mostly a blow on the occiput. Taking everything into consideration, the audiogram produced by a skull injury may vary considerably Indeed, *Manzoni* and *Arcelli* (1957) stated that there is no typical audiogram for skull trauma.

Skull injury mostly produces an inner ear lesion, but the middle ear may also be damaged. Middle ear lesions usually originate from fracture of the temporal bone but the middle ear may be damaged also by other types of skull trauma. *Sakai* (1911) published studies of auditory ossicles damaged as a result of a skull injury Many in estigators today have established lesions in the middl ear especially in the auditory ossicles, in connection with skull traumas. These injuries are manifested most commonly as displacements of the long process of the incus, but every now and then fractures of the auditory ossicles, above all the stapes, are also encountered. Ruptures of the ligaments of th auditory ossicles are also common (*Robinson* 1961 *Arragg* and *Paparell* 1964 *Hammond* 1964 *Does* and *Bottema* 1965, *Kley* 1966).

E er since the beginning of the 20th century the pathoanatomical reasons ha been sought for deafness of inner ear type caused by skull injuries. Opinions h differed ppreciably Most studies ha been on experimental animals, but temporal bone from dead human beings has also been used, bone from men nd women who had sustained during lif a skull trauma resulting in permanent deafness. The inadequacy of the methods used has certainly contributed to the di ergent investiga tion results. Modern methods using electron microscopic studies could h ve given more reliable and consistent results.

Hemorrhages into the perilymph were regarded by Nager (1907) and M ance (1910) as the primary reason. Connective tissue and bone origin ted in consequence of the bleeding. They regarded the degenerative changes in Corti s organ as the result of this connective tissue and bone formation. *Stenger* (1909) established hem rr hages in the scala tympani, especially in the vicinity f the round window He encoun tered hemorrhages also in the cochlear and vestibular nerve. The histologic finding by *Theodore* (1910) was a prevalence of atrophic-degenerative changes in the ner elements, first and foremost in the spiral ganglion. Corti's organ was less dam ged M ny in estigators have considered laceration of nerve fibres f the eighth cereb l ner to be a probable sequela f trauma (*Ulrich* 1926, *Grove* 1939 *Escl er* 1948)

Damage to the finer labyrinthine structure was considered to be a primary eve t l *Wittmaack* (1932). He established the most severe changes in Corti s gan h tl f the second turn, whereas severe lesions were more rare in the region of the b turn. *Wittmaack* believed that the changes were caused by pressure originates in th l byrinthine fluid.

The dam ging effect of labyrinthine fluid which is suddenly set in mo suggested by *Schuknecht Neff* and *Perlmann* (1951) They noted onl hair cells in mild cases in their experiments on cats. Destruction o degeneration of Deiter cells were seen in cases f medium severity t tally destroyed in th most severe cases. Animals decap tated onl the traum displayed degeneration f nerve terminal which was animals sacrificed immediately after the trauma The mo encountered in the upper part of the basal turn which corre frequency This theory was considered correct also by *Proctor* It has in fact gained the most support today

A great many investigators attribute sensori neural deafness most commonly to the traumatisation of an end organ or auditory nerve. Of course, a more central affection may also be involved. *Zange* (1915) for example believed that a skull injury may lead to a hearing defect without peripheral trauma in consequence of damage to the cochlear nucleus or more central nerve fibres. He had encountered traumatic hemorrhages in war injured most frequently just in the brain stem region. Lesion of the brain stem was regarded by *Kecht* (1965) too as a potential reason for hardness of hearing of inner-ear type. However it has been contended in recent times that lesions of the central nervous system often do not exert any appreciable effect on the hearing threshold, but that they do affect speech discrimination (*Fowler et al.* 1966, *Benitez et al.* 1966)

The lesion in a sensori-neural hearing defect may in any case be located at a different level It may be found in the end organ, in the spiral ganglia, the nerve trunk, cochlear nuclei or central fibres from nuclei to the auditory cortex.

Dix Hallpike and *Hood* (1948) came to the conclusion that positive recruitment is a sign of a lesion of Corti's organ. Present research concurs with this view *Fowler's* test is regarded today as the most reliable test for distinguishing between a cochlear and retrocochlear lesion. Partial or complete recruitment is established usually in end organ lesions, while absence of recruitment suggests a retrocochlear lesion. However *the use of Fowler's tests is considerably limited by the condition that the hearing* threshold difference between the ears must be sufficiently great and that the fellow ear must not be deaf.

Nerve lesions often produce a discrepancy between the threshold for pure tones and the threshold for speech discrimination, in other words the speech threshold is not proportionate to the threshold for pure tones. Discrimination score deficiency is also encountered in these lesions as a rule, that is the patient's maximal discrimination score does not amount to 100 per cent. Discrepancy is usually not encountered in end organ lesions and the patient's discrimination score is good (*Harbert* and *Young* 1964 *Fowler* and *Altmann* 1966, *Benitez et al.* 1966, *Eschel et al.* 1966)

Adaptation means the temporary change of the hearing threshold to a new level as the result of tonal stimulation. Pathologic adaptation has been taken as a sign of different diseases. Some claim that it signifies end-organ deafness, others that it denotes a hearing impairment of nervous origin or a central hearing defect. However most investigators today consider it to be an indication of hearing deficiency of the eighth cerebral nerve or a central hearing d fect. For instance, *Benitez et al.* (1966) generally established marked adaptation in lesions of the first neuron and the nerve trunk. They also demonstrated moderate adaptation in both ears in brain stem lesions. Similarly they noted moderate adaptation in the contralateral ear in auditory cortical lesions. It was also reported by *Palva* (1964) and *Palva* and *Palva* (1966) in their studies that pathologic d ptation was much more rare in end-organ diseases than in central lesions and those of the eighth cerebral nerve. However they encountered moderate adaptation in both cochlear and retrocochlear lesions, though not of diagnostic significance. Hence too categorical conclusions should not be drawn from the degree of adaptation. Traumatisation of the corpus trapezoideus of the brain stem can cause

bilateral hardness of hearing. Lesions higher up the brain stem or in the midbrain may cause hearing loss in the contralateral ear. This applies especially to lesions of the lemniscus lateralis. The hearing defect is manifested chiefly as a deterioration of speech discrimination. Pure-tone hearing function is usually preserved. The threshold for pure tones has been found to remain normal also in auditory cortical lesions. The principal auditory impairment encountered in lesions of the central nervous system appears to occur in speech discrimination, localisation of the voice and auditory images (*Fowler* and *Altmann* 1966, *Benitez et al.* 1966).

The localisation of a trauma usually requires several tests. *Fowler's* test is regarded as the most reliable, although it too has been found to be positive in 1–2 cases out of 10 in retrocochlear lesions (*Portmann* 1964). In addition to the methods already mentioned, *Békésy* audiometry with both continuous and interrupted sound is employed fo localisation of the lesion (*Jerger* 1962). Another is the SISI test in which the patient's ability to perceive small changes in intensity is tested. Perception of small intensity changes is often associated with cochlear diseases (*Jerger* 1962).

No direct relationship has been established between the severity of the skull injury and the neuro-otological finding (*Kecht* 1965). Fractures of the temporal bone, however constitute something of an exception. They almost always damage hearing. About 80 per cent of temporal bone fractures are longitudinal (*Schuknecht* and *Davison* 1956). Most of them originate from a blow to the temporal or parietal region. The fracture line passes along the anterior margin of the pyramid and then across the roof of the middle ear or the mastoid cells. The tympanic membrane is often ruptured and there is bleeding from the ear. Displacement of the incus, fracturing of the auditory ossicles and laceration of the ligaments are sometimes seen in the middle ear. A deformity develops occasionally in the external auditory canal. Facial paresis occurs in less than 25 per cent of the cases. Conductive hearing loss originates from injury to the tympanic membrane and middle ear. *Schuknecht* (1950) and *Proctor et al.* (1956) found, however that mere conductive hearing loss was rare in a fractured ear. There is always also some degree of sensori-neural deafness. Some sensori-neural hearing loss is often established in the contralateral ear as well. As regards improvement of hearing, recovery of middle-ear type hearing loss is often good. For sensori-neural hearing loss the prognosis is the same as for other inner ear lesions caused by skull injury. Mild cases showing only a minor notch at 4000 Hz may be cured. A great proportion, however remains stationary and makes no progress (*Müller* 1965).

Transverse fractures of the temporal bone are much less common and generally originate from a trauma to the occipital region. The fracture line runs at right angles to the pyramid via the vestibule and th internal acoustic meatus. The facial nerve is injured in c. 50 per cent of the cases and there is usually damage in the region of the ganglion geniculi. There is often haemotympanum and discharge of cerebrospinal fluid. The membranous labyrinth is destroyed completely by the fracture. The consequence is complete extinction of both hearing function and vestibular function. The material reported by *Grove* (1939) comprised 17 transverse fractures with complete hearing loss in the fractured ear in all the cases. In addition, considerable hearing loss of inner ear type was established in the contralateral ear of 65 per

cent of the patients. *Proctor* and his co-workers (1956) described a series of the same size of transverse fractures: homolateral deafness was noted in every case. Further a sensori-neural hearing deficiency was demonstrated in the contralateral ear in 50 per cent.

Thus, both cochlear and vestibular function are usually extinguished in transverse fracture of the temporal bone. However *Klingenberg* (1929) described two cases in which a separate fracture was established in the cochlea, causing deafness in that ear but vestibular function was preserved. *Schlittler* (1936) reported quite the reverse, a case of a separate fracture of the vestibular organ. It caused complete extinction of vestibular function, but some hearing function was preserved. Cases like this are very rare.

Ringing in the ears or tinnitus is often a subjective symptom following skull trauma. Tinnitus is usually the result of injury to an end organ. *Gurdjian* and *Webster* (1958) reported that 30—40 per cent of the patients with cochleovestibular symtoms as sequelae of skull injury complained of tinnitus. Some patients have continuous tinnitus which may become a very disturbing symptom.

B Vestibular Damage

A high incidence of vestibular lesions resulting from skull injury has been reported. Often, it is even higher than the incidence of hearing damage. For example, the study by *Lumio* and *Aho* (1962) on skull traumas revealed a clearly higher incidence of vestibular than of hearing damage.

Vestibular damage may be peripheral or central. The central portion comprises the four nuclei in the fundus of the fourth ventricle and their connecting fibres to the other centres, above all to the cerebellum, the cortex of the posterior part of the temporal lobe, the optic nerve nuclei of the brain stem and the motor centres of the spinal cord. Scarpa's ganglion in the internal acoustic canal is usually regarded as the boundary between the central and peripheral part.

Windle et al. (1944) stated on the basis of their experiments on pigs that central vestibular lesions may originate as the result of skull trauma. They demonstrated pathologic changes in the fundus of the fourth ventricle. All blows caused chromatolysis in the neuron of the lateral vestibular nucleus. It was believed by *Proctor et al.* (1956) that a peripheral vestibular lesion may originate as the direct consequence of a pressure wave of the labyrinthine fluid or as the result of bleeding. However they regarded damage to the nuclei and central nerve fibres as a more probable cause. *Piquet* and *Piquet* (1958) also held that the majority of the cases involved a central vestibular trauma which would be caused by direct injury to the brain stem or one produced indirectly by circulatory disorders. Similar results were published by *Pirodda* and *Cenacchi* (1964). *Lehnhardt* (1965) reported that central vestibular lesions are much more common than central hearing damage in blunt skull injuries. Contradic-

ting this, animal experiments have shown that a mere cortical lesion does not influence estibular function provided that the other parts of the brain were protected against damage (*Hofman* 1961).

The origination of a peripheral vestibular injury is axiomatic when a fracture of the bony labyrinthine capsule and destruction of the membranous labyrinth are involved. This is the case in transverse fractures of the temporal bone. The most probable reason for a peripheral trauma in other cases is held to be the sudden setting in motion of the labyrinthine fluid. The same thing is believed to cause peripheral hearing damage. Separation of the otolithic membrane from the utricle and saccule has been demonstrated in histologic studies of the damaged vestibular end organ. Shallowing of sensory epithelium has been seen in the maculae of the utricle and saccule as well as in the crista ampullaris. Nerve fibres are degenerated later (*Nassulphus* 1946, *Proctor* et al. 1956)

Vertigo is a very common complaint by patients with skull injuries. Fifty per cent of the patients had vertigo in the material described by *Gurdjian* and *Webster* (1958) In *Ey*'s series (1966) the percentage was 61 *Shambaugh* (1967) defined vertigo as a sensation of rotation in which either the patient is turning round or surrounding objects appear to be spinning round him. It originates from both peripheral and central vestibular lesions. The sensation of weakness caused by cerebral ischemia must be distinguished from true vertigo

Vertigo of peripheral etiology is usually manifested as short episodes of rotatory vertigo. This condition is often accompanied by spontaneous nystagmus. Symptoms emanating from the autonomous nervous system, such as nausea and vomiting, are common. Destruction of the vestibular end organ occurs in connection with transverse fracture of the temporal bone. Vertigo associated with it represents in fact typical peripheral vertigo. It usually disappears in a couple of weeks. The transient nature of the symptoms is possibly due to the ability of the vestibular nuclei to compensate the disturbed impulses departing from the damaged labyrinth (*Gurdjian* and *Webster* 1958) The sense of sight and sense of touch also play an important part in the compensation process. On the other hand, central compensation occurs alongside the slow gradual destruction of the vestibular organ, and the symptoms remain slight. Hearing loss associated with vertigo generally indicates a peripheral etiology (*Jongkees* 1965) The uditory and vestibular pathways meet in a small area in the region of the corpora quadrigemina, and if the lesion is localised in this tract other neurologic symptoms are also involved.

A lesion of the nuclei and central fibres generally does not cause episodic rotary ertigo the disturbance of equilibrium is more diffuse (*Jongkees* 1965) Nor is ertigo of central origin commonly as intense as vertigo caused by an end organ (*Altmann* 1964) It is, however often difficult to infer the origin of vertigo from its character Peripheral and central factors may lie concurrently behind the symptoms f vertigo

Spontaneous nystagmus is one of the main symptoms of a estibular disturbance. Its incidence in connection with skull trauma depends, naturally on the type of injury in question. Transverse fracture f the temporal bone causes sudden destruction

of the vestibular end organ. Spontaneous nystagmus almost always occurs in such cases. *Lange* and *Kornhuber* (1962) established spontaneous nystagmus in 11 per cent of patients who had sustained cerebral concussion. Spontaneous nystagmus is generally indicative of a pathologic condition. Some investigators, however hold that it may sometimes occur also in healthy people. For instance, *Jongkees* and *Philipszoon* (1964) established weak spontaneous nystagmus in a normal series in the ratio 1:85.

Both a peripheral and a central lesion may be the etiology of spontaneous nystagmus. Horizontal rotatory nystagmus is considered to suggest a peripheral etiology In contrast, many investigators regard purely horizontal, vertical or rotatory nystagmus as indicating a central lesion (*Van Egmond et al.* 1949 *Jongkees* 1965) It has been sought to determine the origin of nystagmus from its direction. The direction of peripheral spontaneous nystagmus is generally towards the healthy ear However *Jongkees* and *Philipszoon* (1964) are of the opinion that it is not possible in peripheral lesions to decide the injured side from the direction of spontaneous nystagmus.

Peripheral spontaneous nystagmus generally disappears in a few weeks. Spontaneous nystagmus of long duration is regarded by the majority of investigators as a sign of a central vestibular lesion (*Van Egmond et al.* 1949 *Altmann* 1964 *Cabersek* and *Jobert* 1965, *Busis* 1965)

Spontaneous nystagmus of peripheral origin is often accompanied by severe vertigo The vertigo is considerably milder or absent in connection with spontaneous nystagmus of central origin (*Altmann* 1964 *Busis* 1965)

Impaired hearing and tinnitus are often associated with peripheral spontaneous nystagmus. Normal hearing and spontaneous nystagmus suggest a central lesion (*Altmann* 1964 *Busis* 1965 *Fowler* and *Altmann* 1966)

Post traumatic spontaneous nystagmus is often difficult to establish by mere inspection. *Pirodda* and *Cenacchi* (1964) observed that spontaneous nystagmus established a long time after the trauma is often of low amplitude and difficult to diagnose

Lange and *Kornhuber* (1962) stated that spontaneous nystagmus may at intervals become latent spontaneous nystagmus, which means that spontaneous nystagmus would not be established at every examination and that e.g. stimulation of the central nervous system, psychic excitation, physical strain and especially irritation of the vestibular organ provoke spontaneous nystagmus.

Postural nystagmus refers to nystagmus which occurs only in a certain position of the head or to nystagmus that is decisively connected with the position of the head. It is often noted after skull injuries. *Cawthorne* (1954) considers skull trauma to be the most common etiological f ctor in postural nystagmus. *Proctor et al.* (1956) demonstrated postural nystagmus in 25 per cent of their skull injury series. As high a percentage as 50 was reported by *Pfaltz* (1956) He found that it disappeared fairly rapidly post traumatically and rarely remained a permanent condition. *Barber* (1964) noted postural nystagmus in 25 per cent of his material. It was twice as common in skull fractures as in the cases with no skull fracture He found that a longitudinal fracture of the temporal bone caused postural nystagmus especially frequently (47 per cent)

Some authors have taken postural nystagmus to be a lesion caused by otoliths (*Dix* and *Hallpike* 1952, *Cawthorne* 1954). *Vogel* (1931) attributed it to the semicircular canals. *Miehlke* (1952) ascribed peripheral postural nystagmus to injury f the semicircular canal. *Vogt* (1954) believed traumatic postural nystagmus to be of central origin, caused by circulatory disturbances due to trauma. It is held today that postural nystagmus may be caused by both peripheral and central factors (*Frenzel* 1961, *Henriksson* 1966)

An endeavour has been made to localise the trauma on the basis of the direction of postural nystagmus. Three different types of postural nystagmus were distinguished by *Nylén* (1958) direction-changing (type 1), fixed-direction (type 11) and irregular postural nystagmus (type III) He considered types I and III to indicate a central lesion, type II a peripheral lesion. *Proctor et al.* (1956) also stated that peripheral vestibular injury causes postural nystagmus which has a static, fixed direction. If the nystagmus changes direction, a disturbance must be suspected in the vestibular nuclei or central pathways. However *Litton* and *McCabe* (1966) among thers, believe that the direction of nystagmus or a change in its direction give no indication of the localisation of the lesion.

The duration of postural nystagmus is now considered to play an important role in the localisation of a lesion. A latent period is characteristic of transitory postural nystagmus. The duration of the nystagmus is also limited. *Lindsay* (1951) considered 60 seconds to be the limit. A feeling of vertigo usually accompanies the nystagmus. Another typical feature of this nystagmus is that it cannot be provoked repeatedly Transient postural nystagmus is generally held to signify a peripheral lesion. Persistent postural nystagmus suggests a central injury (*Frenzel* 1961 *Busis* 1965, *Litton* and *McCabe* 1966) This assumption is reinforced by the circumstance that persistent postural nystagmus without a latent period is often noted in diseases of the central nervous system (*Cawthorne* 1954).

Normal hearing associated with postural nystagmus suggests a central lesion (*Gurd nan* and *Webster* 1958, *Milojevic* 1967)

The most important part of the vestibular examination is the caloric test by means of which the ears can be examined separately Electronystagmography which is based on the registration of the electric impulses which originate in nystagmus, is used now for objective recording of nystagmus. Man has positive electric potential in the cornea and a negative one in the retina. The bulbar movements which are produced in nystagmus cause variations in potential. The electric impulses are led via electrodes and a amplifier into the electronystagmograph which records the result. The method has the great advantage of registering even slight nystagmus that might otherwise escape detection. *Jongkees* and *Philipszoon* (1964) found it impossible to detect nystag mus under 7 deg by inspection, but nystagmus of 2–5 deg. was easy to observe elect ronystagmographically As the examination is performed with the patient keeping his eyes closed, the effect of fixation of gaze on the reaction is a oided. Various parameters such as duration, velocity of the slow component, latency total amplitude total frequency and total number of strokes can be calcul ted from the curve obtained. The best possibl result is achieved by using different parameters (*Gulick* and *Pfaltz* 1964)

Rotatory nystagmus does not cause fluctuations in potential and cannot be registered. The functions of the vestibular organ can be evaluated from the electronystagmogram with the help of parameters. Especially the duration and the speed of the slow component are important (*Aschan et al.* 1956 *Stahle* 1958) Latency is no longer held to be of any great importance in assessing the result of the caloric test (*Riesco-Mac-Clure* 1964)

Unilateral vestibular weakness has been held to point to peripheral lesion (*Milojevic* 1967) *Gulick* and *Pfaltz* (1964) found that the intensity of the reaction always decreases in peripheral lesions. They stated that intensification of the reaction is proof of a central lesion.

Lumio and *Aho* (1962) used an electronystagmograph for vestibular studies on patients diagnosed as having post traumatic encephalopathy They observed that the nature of the vestibular disturbance varied. The commonest type of reaction in their series was one in which the variation of both the amplitude and the frequency of the nystagmus was quite irregular There were also many cases in which the caloric test elicited no reaction at all

The result of the caloric test is sometimes dysrhythmia i.e periodic nystagmus. Intensive nystagmus and a latent period alternate. This is considered to indicate central vestibular lesion. *Aschan et al.* (1956) noted that dysrhythmia sometimes occurred after skull injuries. They also found that electroencephalography revealed disturbed electric activity of the brains in every case of electronystagmographic dysrhythmia. The disturbance was mostly localised in the temporal lobe. In their experiments on cats, *Fredrickson* and *Fernandez* (1964) concluded that both dysrhytmia and vestibular hyperactivity might be caused by the liberation of vestibular nuclei from cerebellar inhibition.

Alternating nystagmus is a very rare vestibular disorder The direction of the nystagmus varies. Skull injury has been found to be the etiologic factor in at least a part of the cases (*Gramowski* and *Unger* 1965)

Directional preponderance means that reaction develops more readily in one direction than another Cold water calorisation of e.g the left ear and hot water calorisation of the right ear provokes a stronger reaction than cold water irrigation of the right ear and hot water irrigation of the left ear Thus, both cold and hot water calorisation are necessary to demonstrate th phenomenon. *Fitzgerald* and *Hallpike* (1942) held that directional preponderance was a manifestation of central vestibular lesion. They observed directional preponderance to the side f the lesion in all temporal lobe injuries. Since then, however many workers have found that directional preponderance may be associated also with peripheral traumas (*Hart* 1965, *Coats* 1965) *Gulick* and *Pfaltz* (1964) too, mentioned that directional preponderance may be associated with peripheral injuries as well, although this is not common. It was reported by *Coats* (1965) that spontaneous nystagmus to the side of the healthy ear in peripheral vestibular injuries can gradually change into directional preponderance to the same direction. He reported an incidence f 17.5 per cent for directional preponderance in a normal material. *Mehra* (1964) mentioned however that directional preponderance is not general in a normal material, nd he reported an incidence of only 5.2 per cent.

As mentioned, normal hearing in connection with vestibular symptoms such as spontaneous or postural nystagmus is regarded as evidence of central vestibular lesion. The association of bilateral vestibular lesion with normal hearing is also taken to denote a trauma in the estibular nuclei (*Ruesco-Mac-Clure* 1964)

C War Injuries

There are a few hearing and vestibular studies from World War I dealing with persons wounded in the head (*Goerke* 1924) But they are very imprecise because investigation methods t that time were still inadequate.

Audiometric studies were conducted during World War II and revealed numerous hearing defects. Head injuries usually caused deafness of inner ear type (*Siirala* 1945) *Hormia* (1959) observed that audiograms of war-injured usually showed a declining type of curve. The decline was recorded at 4000 Hz in third of the cases.

Apart from head wounds, acoustic traumas and hearing damage caused by explosion must be borne in mind as war time factors causing hardness of hearing. Their great role in the etiology of hearing defects was established clearly during World War II (*Siirala* 1945)

Hearing damage caused by detonation causes chiefly a unilateral sensori-neural hearing deficiency which is often manifested as a C5 notch in the audiogram. The tympanic membrane is usually not ruptured. Detonation damages chiefly the external hair cells and the recruitment phenomenon is therefore positive on the whole. Nerv fibres and the cells f the ganglion spirale re damaged only after the internal hair cells are already destroyed (*Lehnhardt* 1965)

The pressure peak is longer in explosion than in detonation damage The hearing defect is mostly bil teral. Rupture of the tympatic membrane is common. It was observed during the war that damage caused by explosion resulted in a much more severe cochlear lesion if there was no rupture f the tympanic membrane (*Kecht* 1964) If the tympanic membrane remains intact, all the energy passes into the inner ear through the chain of auditory ossicles. When the tympanic membrane ruptures, the pressure is exerted simultaneously on both windows, the energy entering the inner ear is smaller and the damage to it is less severe. The deafness caused by explosion is combined hearing defect. Damage to the tympanic membrane and the middl ear causes a conductive hearing loss. Sensori-neural hearing impairment was manifested in the audiogram as a depression t the high tone level the hearing deficiency is often also pancochlear (*Lehnhardt* 1965)

III PURPOSE OF THE INVESTIGATION

The object of the present investigation was to establish the incidence and nature of hearing and vestibular defects in 256 brain injured ex servicemen on the basis of a neuro-otological study

Additionally the aim was to

(1) study the proportion of peripheral and central lesions in the material and

(2) seek possible correlations between the neuro-otological finding and localisation of the trauma.

IV MATERIAL

The material consisted of 256 patients who had been wounded in the Finnish wars of 1939–1940 and 1941–1944. The investigation was conducted at the Otolaryngological Hospital, University of Helsinki, in 1965–1966. The interval between the occurrence of the trauma and the investigation was thus c. 25 years on an average. The patients were referred for examination by the Institute for the Rehabilitation of Brain Injured Veterans (Sotainvalidien Veljesliiton Aivovammaisten Huolto- ja Tutkimuslaitos). This is a neurological clinic which specializes in the examination and treatment of ex-servicemen who sustained head injuries during the war. The patients stay in hospital a few weeks and are called for follow-up studies at regular intervals. The only criterion followed in selecting the material was that all the patients had a brain injury diagnosed at neurologic examination at the Institute for the Rehabilitation of Brain Injured Veterans. The series was otherwise unselected. It is not, however, perhaps fully representative of a wartime brain injury material as patients who were under examination in the Institute at the time were included in the study for practical reasons.

The *age* of the patients at the time of *wounding* is given in Table 1.

TABLE 1 – *Age when wounded*

Age	Number of cases	Per cent
under 20 years	17	6.6
20–24 years	79	30.9
25–34 years	128	50.0
over 35 years	32	12.5
Total	256	100.0

The above age classification was employed by *Hillbom* (1959) in his studies of brain injured ex-servicemen.

Fifty per cent of the patients in the present series were wounded between 25 and 34 years of age. Patients under 20 years (6.6 per cent) constituted the smallest group. Patients over 35 accounted for only 12.5 per cent.

The cause of the wound was a splinter in 143 cases (55.9 per cent), a bullet in 59 cases (23.0 per cent). In 54 cases (21.1 per cent) the cause was some other factor such as the patient being blown over by the air pressure from an explosion, traffic accident, or a fall, mostly from a moving vehicle.

The localisation of the injury was decided according to the site of impact, the information available on the mechanism of the trauma and the neurologic findings. The localisation of the injury is shown in Table 2.

TABLE 2 – *Localisation of the injury*

Localisation	Number	Per cent
Parietal traumas	65	23.1
Frontal traumas	61	21.7
Temporal traumas	48	17.1
Facial traumas	23	8.2
Occipital traumas	22	7.8
Concussion-contusion injuries impossible to localise more precisely	62	22.1
Total	281	100.0

The trauma had directly affected the aural region in eight of the 48 cases with a temporal injury. It was not possible to decide the localisation in 62 cases (22.1 per cent). In most of these cases the injury was caused by another agent than a splinter or a bullet. A total of 219 different localisations were established in 194 cases. In other words, an individual patient may have had several localisations.

Type of injury. The wound was open in 112 cases (43.8 per cent) and closed in 134 cases (52.3 per cent). Open injuries refer to traumas which penetrated the cerebral tissue causing both fracture of the bone and laceration of the dura. It was impossible in 10 cases (3.9 per cent) to decide whether the injury was open or closed.

Table 3 gives the data on *primary unconsciousness* due to the trauma and its duration.

TABLE 3 – *Primary loss of consciousness and its duration*

Unconsciousness	Number of cases	Per cent
No loss of consciousness	57	22.3
Unconscious for under 1 hour	66	25.8
Unconscious for 1–24 hours	39	15.2
Unconscious for over 24 hours	29	11.3
History of primary loss of consciousness but no information as to its duration	30	11.7
No information on primary loss of consciousness	35	13.7
Total	256	100.0

Primary unconsciousness following the trauma occurred in at least 164 cases (64.0 per cent). No primary loss of consciousness was caused in 57 cases (22.3 per cent). The result is in good agreement with *Hillbom's* (1959) findings. There was no loss of

consciousness in 23.6 per cent of his material f brain-injured ex-servicemen. Twenty nine patients (11.3 per cent) were unconscious for over 24 hours. The corresponding percentage in *Hillbom*s material was 10.2.

Primary rev sion was performed in 138 patients (53.9 per cent) Head operations were performed later on 37 p tients (14 4 per cent) The main indications for surgery were removal of foreign body drainage of a focus of infection and pilepsy In some cases a plastic operation was performed for an extensive skull defect.

The injuries were divided into the following *sev ity groups* on the basis of the invalidity degree set by the State Casualty Office mild (invalidity under 30 per cent) medium (invalidity 30—65 per cent) and severe (in alid ty 70—100 per cent) The severity grading for each patient was taken from the case reports of the Institute for the Rehabilitation f Brain Injured Veterans. Th distribution according to invalidity is, naturally open to argument, but it probably gives some idea of the severity of the injuries in question. Mild traumas in the material totalled 69 (27.0 per cent) traumas of medium severity 148 (57.8 per cent) nd severe traumas 39 (15.2 per cent) Traumas of medium severity were thus in the majority In *Hillbom* (1959) basic material f brain-injured ex-servicemen (3552 cases) mild traumas ccounted for 18.8, medium severe for 59 3 and severe for 21.8 per cent. The number f mild traumas in the present series was greater than in *Hillbom*s material (27 0 per cent > 18.8 per cent) and th t of severe traumas smaller (15.2 per cent < 21.8 per cent) This may be because some of the severely injured patients had already died or were under continuous institutional treatment, leaving fewer who attended the Institute for the Rehabilitation of Brain Injured Veterans in recent years.

T aumat ep l psy developed in 87 cases (34 0 per cent) It was established in 50.9 per cent of open and 18 6 per cent f closed injuries. These figures concur well with those presented in the literature. *Gurdjian* and *Webster* (1958) reported the incidence of traumatic epilepsy in peacetime injuries to be 15—22 per cent and in wartime traumas 18.9—45 per cent. The incidence is generally much lower in closed than in open wounds. Epilepsy was established in 30.4 per cent f *Hillbom*s basic material f brain-injured ex-servicemen the ratio was 44.2 per cent for open and 20.3 per cent for closed wounds.

Monopa esi or *h mipa is* was established post-traumatically in 49 cases (19 1 per cent) *Aphas a* was present in 17 cases (6.6 per cent) Recovery from phasia was generally excellent. The prognosis for patients with traumatic phasia is generally good (*Gurdjian* and *Webster* 1958 *Hillbom* 1959)

El ct oencephalography was perf rmed on 252 patients (98.4 per cent). The finding was normal in 86 cases (34 1 per cent) Diffuse dysrhythmia wa established in 114 cases (45.2 per cent) and focal dysrhythmia in 52 cases (20.6 per cent) This mode f distribution was used by *Hillbom* (1959)

Pn um nc phal g aphy was performed on 170 patients (66 4 per cent) The finding was normal in 57 cases (33 5 per cent) and pathologic in the rem ining 113 (66 5 per cent)

V METHODS OF INVESTIGATION

A Early Symptoms

Early symptoms refer to aural symptoms such as bleeding from the ear suppu ration, hardness of hearing and vertigo established immediately or soon after being wounded. The data are based on old field hospital and military hospital case reports which were, understandably often rather incomplete.

B Present Symptoms

The patients were asked to state their own opinion of the present state of their hearing. They were also asked about the presence of tinnitus. They were asked to try and distinguish between actual ringing in the ears and a sensation of humming in the head. The occurrence of disturbances of equilibrium was inquired about. Attention was also paid to their nature. The patients were also asked about headache and nausea. However only more frequent than normal headache was considered, that is almost daily or constant headache.

C Otoscopy and Clinical Inspection of the Nose Mouth Pharynx Nasopharynx and Larynx

The initial examinations were otoscopy and clinical inspection of the nose mouth, pharynx, nasopharynx, and larynx.

D Audiologic Studies

As the object was to ascertain the incidence of hearing and vestibular d fects and the proportion of peripheral and central lesions in the material, in analyzing the results th material was not divided into groups according to the type of hearing deficiency but treated as a whole.

Mads n and *Beltone* audiometers were used, calibrated according to the NPL standard A pure tone audiogram with air and bone conduction threshold determinations was taken from all the patients. Hearing defects were distributed into conductive sensori-neural and combined defects.

A pure tone audiogram in which the hearing threshold was in the frequency range of audiometer 20 dB t the most was considered normal

The average hearing loss was calculated as the mean of the hearing thresholds at 500, 1000 and 2000 Hz.

Although the actual upper limit of hearing was not determined, the highest frequency heard by the patient was determined from the pure tone audiogram to an accuracy of one octave.

The shape of the hearing curve was also obtained from the pure tone audiogram. The starting point of the depression in the hearing curve was taken as the frequency from which the curve was depressed for a minimum of 10 dB over the range of one octave. A hearing curve in which the hearing loss was fairly even in all the frequencies was interpreted as horizontal.

Speech audiometry was also performed on all the patients. The threshold for speech discrimination was determined first, i.e. the intensity of the tone at which the patient heard a half of the 10 test words correctly. After that, maximal discrimination score was determined in per cent by increasing the intensity of the threshold for speech discrimination by 30 dB. The speech audiometer was calibrated so that the threshold of a patient with normal hearing for speech discrimination was a maximum of 35 dB.

The mean of the pure tone hearing threshold and the threshold for speech discrimination were compared for possible discrepancy. If the difference between these thresholds was 35 dB or more, discrepancy was considered to exist.

For study of loudness recruitment, *Fowler's* test was performed in all the cases with a definite difference in hearing ability between the ears at the hearing threshold level. The test sound was given alternately into each ear.

The adaptation test was performed by the method introduced by *Palva* and *Palva* (1966) in which adaptation is measured at the threshold level. The subject listens at the 5 dB sensation level to a continuous sound which is amplified in 5 dB degrees when it ceases to be heard. A change in the hearing threshold is established after three min. The test was performed at 1000 and 4000 Hz. However, it could not be performed at 4000 Hz in all the cases because the hearing threshold in this range was so high that adaptation could not be observed for a full three min. Nor could the hearing threshold be measured at 4000 Hz in all the cases. In addition, it was not possible to perform the test on ears with very poor hearing.

The maximum hearing threshold shift recorded at either of the frequencies mentioned was noted.

The possibility of simulated hearing loss was also taken into consideration during the examination. Suitable methods were employed in some cases in order to disclose the simulation.

E General Neuro-otological Tests

Romberg's test, the stepping test and the past pointing test were the common neuro-otological tests employed in the present study.

In *Romberg's* test, a definite tendency to fall was denoted as positive. The stepping test generally involves 50 pairs of steps. Owing to the poor condition of many of the

present patients, however 50 single steps were used. As far as possible the effect of environmental sound or sources of light on rotation were eliminated during the test. The angle of rotation was calculated.

In the past pointing test the patient, eyes closed, held his arms stretched straight out and moved them in the sagittal plane with the extended fingers pointing at the examiner's extended fingers. Past pointing was considered to be involved when the deviation of the hands was at least 5 cm in two past pointing tests (*Nylen* 1958)

F Vestibular Special Studies

1 SPONTANEOUS AND POSTURAL NYSTAGMUS

Spontaneous nystagmus was studied by inspection without Frenzel's spectacles, with the patient looking straight ahead and then fixing his gaze to each side in turn. In addition, spontaneous nystagmus was studied and recorded by electronystagmography before the caloric test with the patient's eyes closed and then gazing to each side in turn.

Postural nystagmus was ferified by inspection. The patient was placed on his back and his head was turned from one side to another The same turning of the head was repeated with the head hanging down. *Frenzel* (1961) found that this simple method of examination sometimes gives an even clearer idea of postural nystagmus than moving the patient as a whole. The same conclusion was reached by *Cawthorne* (1954)

2. CALORIC TEST

The caloric test was made according to the method of *Fitzgerald* and *Hallpike* (1942) using both 30°C and 44 C water but with 30-second instead of 40-second calorisation. It has also been used by *Aschan et al.* (1956) Stronger calorisation, e.g. iced water was not used because it might have strained these patients excessively and provoked an epileptic attack in some of them. The water used in the calorisation process was taken from a thermostatically controlled vessel, accuracy ± 0.2°C.

Instead of water calorisation, 60-second air calorisation with air of room temperature was performed in cases with dry perforation of the tympanic membrane. A 5-minut interval was observed after each calorisation. The patient lay on his back during the test *with his head bent c. 50 deg forward. The horizontal semicircular* canals are in the vertical plane in this position. The calorisation was performed in a semi-dark room. The patient's eyes were closed during the procedure The result of the caloric test was recorded by electronystagmography Concave electrodes, 10 mm in diameter filled with electrode paste were fixed to the patient's temples close t the lateral corners of the eyes. Electric impulses were led via an lternating current preamplifier into the electronystagmograph which recorded the result on paper tape A 1-channel ELEMA mingograph was the electronystagmograph used. The time

constant was two seconds. Th speed of the paper was 12 5 mm/sec. It was possible t register only horizontal nystagmus with this method. Calorisation was preceded by calibration during which the patient fixed his eyes in turn n two lamps placed at an angle of 10 deg

The period of latency duration and maximum intensity were calculated from the different parameters.

For determination of the latent period attention was paid to the time f onset of the reaction and whether t began during calorisation or onl after t.

Latency was determined only by cold water and air calorisation.

The duration was measured in seconds from the onset of reaction *Ormerod* (1963) stated th t it is often difficult to observe the time of termination of nystagmus for the amplitude and frequency of nystagmus become irregular towards th end. A series of after nystagmus may also appear For this reason attention was paid in the present study to whether the termination of the reaction could be fixed accurately Maximum intensity refers to th speed of the slow phase of nystagmus when the reaction is at its maximum, which happens generall 60—90 sec. fter the beginning of the calorisation. Maximum intensity was calculated according t *Stahle* (1958, 1964) The amplitudes of all strokes were dded together for 10 sec. cycle at the maximum range of the reaction and the length was con erted to degrees on the basis f cal bration. The result was expressed in degrees per second. The standard deviation of the maximum intensity is greater than the duration. Ten per cent is taken as the permissible difference in duration between the right and left ear and 20 per cent for the maximum intensity (*Aschan et al.* 1956 *Stahle* 1958)

In evaluating the type of electronystagmogram, the amplitude and frequency of nystagmus and their vari tions were noted.

The type of th lectronystagmogram and the parameters mentioned, above ll duration and maximum intensity were taken int consideration in evaluating the calori reaction.

The variations from n rm l for both duration and maximum intensity that ha been reported in the literature are very great and ary with the different investigators. The bilateral-symmetrical reaction is ften extremely d fficult to assess because of th great fluctuations in normal values. Normal estibular reactions in the present material fulfilled the following requirements the electronyst gmogram was regular in type in regard to both ampl tude and frequency the duration was minimum of 80 sec. and maximum intensity at least 5 deg./sec. Thus the requirements for normal reaction were set fairly high.

Fo directional preponderance the requirement was that the difference between th cold water calorisation of the right ea + the hot water calorisation of the left ear and th cold water calorisation of the left ear + the hot water calorisation f the right ear must be over 10 per cent for duration and over 20 per cent for maximum intensity

The following indications were taken as suggesti f a central estibula lesion spontaneous nystagmus of long duration (an average f bout 25 years in the patients of this series) directional preponderance lectronystagmographic dysrhythmia and association of normal hearing with bilateral estibular lesion. Any one of these symptoms was taken to suggest central lesion.

VI RESULTS

A Early Symptoms

It appears from the field hospital and military hospital case reports that a post traumatic otological examination was performed on 75 patients (24.2 per cent) It was done in 39 cases by an otological specialist.

Table 4 shows the aural symptoms established after the wound.

TABLE 4 – *Ear symptoms*

Symptoms	Number of patients	Per cent
Hemorrhage	15	6.1
Discharge of pus	12	4 7
Impaired hearing	80	31.2

Thus, 31.2 per cent complained of impaired hearing immediately after they were wounded. Hemorrhage and discharge of pus from the ear were established objectively F re patients had had both bleeding and discharge of pus from the ea In addition, 104 patients (40.6 per cent) had vertigo, but its nature is not defined in the case reports.

B Present Symptoms

In the inquiry conducted in the present study 170 patients (66 4 per cent) reported that their present hearing was distinctly impaired. Tinnitus was a complaint with 126 patients (49.2 per cent) It was continuous in 43 and intermittent n 83 cases. Sixty seven patients had tinnitus in one ear only 59 bilaterally

Humming in the head was reported by 35 patients (13.7 per cent) Disturbances of equilibrium were mentioned by 183 patients (71.5 per cent) Table 5 shows the type of disturbed balance reported by the patients.

TABLE 5 – *Type of equilibrium disturbance*

Type of disturbance	Number	Per cent
Disturbed balance in sudden movements	151	66.5
Occasional difficulty in walking	46	20.3
Episodes f rotary vertigo	17	7.5
Vertigo of ship-deck type	13	5.7
Total	227	100 0

The commonest type was disturbance of equilibrium on making sudden movements. It can also be seen that the same patient may have complained of several different types balance disturbances.

Unusually frequent headache was the complaint of 213 patients (83.2 per cent) and 89 patients (34.8 per cent) suffered from nausea.

C The Otoscopical Finding and Clinical Examination of the Nose Mouth Pharynx Nasopharynx and Larynx

The otoscopy finding is given in Table 6

TABLE 6 – *Otoscopy finding*

Finding	Number of patients	Per cent
Normal	199	77.7
Perforation f tympanic membrane and discharge f pus from the ear	5	2.0
Dry perf ration or secondary membrane in the tympanic membran	29	11.3
Tympanic membrane intact but cicatrised or atrophic	11	4.3
Adhesive tympani membranes	5	2.0
Ear operated on	7	2.
Total	256	100.0

The otoscopy finding was normal in the majority of the cases (77.7 per cent) Tw per cent of the p tients had perf ration of the tympanic membrane and discharge of pus from the ear These cases in olved chronic, suppurati e middle ear inflammation

Clinical examination f the nose, mouth, pharynx, nasopharynx and larynx revealed nothing noteworthy in bearing w th the exception of one case of congenital harelip and cleft palate.

D Audiologic Studies

T ble 7 shows the mean hearing loss according to the pure tone udiogram

There were no totally deaf patients. In the ther hand, patient with ... totalled 25 (9.8 per cent)

The pure tone audiogram was normal for 79 ears (15.4 per cent) T... udiogram was normal for both ears f 28 patients (10.9 per cent)

TABLE – *Average hearing loss according to the pure tone audiogram*

Mean hearing loss (500–1000–2000 Hz)	Number of cases	
	better ear	poorer ear
20 dB or under	156	89
21–30 dB	62	69
31–50 dB	29	42
51–70 dB	7	20
over 71 dB	2	11
totally deaf	0	25
Total	256	256

Table 8 shows the nature of the hearing defect revealed by the pure tone audiogram

TABLE 8 – *Nature of the hearing defect*

Nature of defect	Number of ears
Conductive	12
Sensori-neural	336
Combined	60
Total	408

The hearing defect was of purely middle ear type in only 12 ears (2.9 per cent) A purely inner ear type of hearing defect was encountered in as many as 336 ears (82.4 per cent)

The numerical distribution of the mean air conduction of the pure tone audiogram (500–1000–2000 Hz) is given in Fig. 1 All 487 ears tested for air conduction are included. In other words, the mean air conduction could be determined for all except the deaf ears. The figure shows that the mean air conduction was in the range 5–25 dB for the majority of the ears. There were relatively few with a mean of over 40 dB

The corresponding percentual accumulation curve is drawn in Fig 2. Roughly 60 per cent of the ears had a mean of 25 dB or better

The numerical distribution of the mean bone conduction of the pure tone audiogram (500–1000–2000 Hz) is shown in Fig. 3 All 468 ears for which the test was possible are included. In other words, bone conduction hearing was so poor in 19 ears although the mean air conduction could be determined that the mean could not be calculated. The distribution resembles that of air conduction, but reveals that particularly good means were definitely more numerous for bone than for air conduction. However a lower mean was accompanied by a distinctly sharper decrease in number than for the patients with a corresponding air conduction mean.

The corresponding percentual accumulation curve is presented in Fig. 4 Almost 90 per cent of the ears had a mean of 25 dB or better

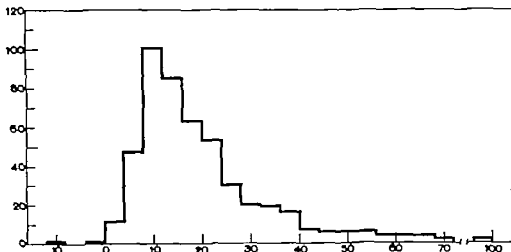


FIG. 1 — *Numerical distribution of th mean air conduction (500–1000–2000 Hz) f th pure tone audiogram. 48? ears. Th number f ears is th ordinat and the mean in decibels th abscissa.*

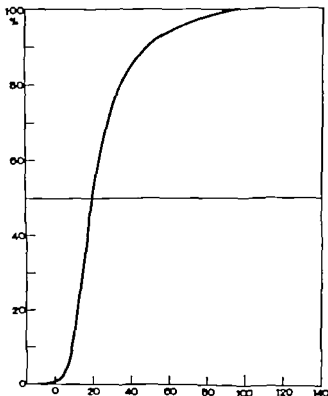


FIG 2 — *Percentual accumulation curve 48? ears. The number of ears in per cent is the ord nat and the mean air conduction in decibels th abscissa.*

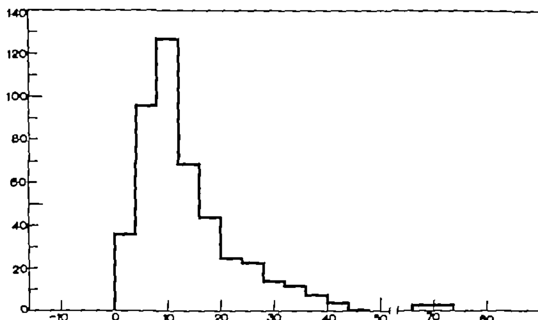


FIG 3. — Numerical distribution of the mean bone conduction (500—1000—2000 H) f the pure tone audiogram 468 ears. Th number f ears th ordinate and the mean in decibel the abscissa.

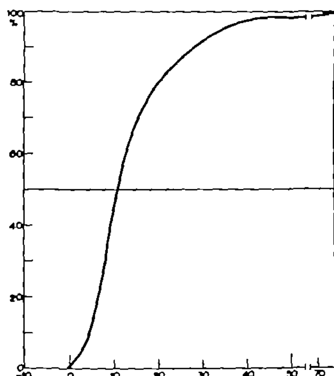


FIG 4 — Percentual accumulation curve 468 ears. The number of ears in per cent is the ordinate and the mean bone conduction in decibels the abscissa.

T ble 9 gives the highest frequency heard by the patients. It was determined to an accuracy of one octa e from the pure tone audiograms.

TABLE 9 – *Highest frequency heard*

Hz	Number f ears
8000	426
4000	42
2000	19
Total	487

All hearing ears were thus capable of hearing a sound at 2000 Hz, and 426 ears (87.5 per cent) heard 8000 Hz.

The shape of the hearing curve of the pure tone udiogram in sensori-neural and combined cases of deafness is given in Table 10.

TABLE 10 – *Shape of the pure tone audiogram*

Shape of udiogram	Number f cases
Horizontal	99
Declining throughout	8
Decline starts from .00 Hz	10
Decline starts from 1000 Hz	62
Decline starts from 2000 Hz	190
Decline starts from 4000 Hz	27
Total	396

The declining type of curve, total 297 (75.0 per cent) was the commonest type These cases included th greatest number f the type in which a depression started from 2000 Hz. They totalled 190 (48.0 per cent) Horizontal curves were 99 in number (25.0 per cent) There were no other types of curve in the material.

The depression of the curve was followed later at the higher frequencies by a new levation in 113 cases (38.0 per cent)

The speech threshold could be determined in 482 ears (94.1 per cent) Table 11 shows the threshold for speech discrimin tion.

TABLE 11 – *Threshold for speech discrimination*

Threshold	Number f cases	Per cent
35 dB or better	186	36.3
40–45 dB	190	37.1
50–65 dB	70	13.7
70–85 dB	24	4.7
over 85 dB	12	2.3
Total	482	94.1

The threshold for speech discrimination was 45 dB or better in 376 cases (73.4 per cent) However the speech threshold was impossible to determine in five cases although a pure tone audiogram was obtained. One ear among these cases heard correctly three out of 10 test words at maximal voice intensity The number of correctly heard words was two in two cases. Only one word was heard correctly in one case, and there was one case in which not a single of the 10 test words was heard correctly

Discrepancy between the threshold for pure tones and the threshold for speech discrimination was established in 24 cases (5.0 per cent) the difference between the thresholds was 35 dB or more No such discrepancy was observed in 458 cases (95.0 per cent)

The discrimination score in hearing ears was established for 451 ears (92.6 per cent) It was thus possible in these cases to add 30 dB to the threshold for speech discrimination. The possible discrimination loss is shown in Table 12.

TABLE 12 — *Discrimination loss*

Discrimination loss	Number of ears	Per cent
No discrimination loss	405	89.8
Discrimination loss 10 / or less	27	6.0
Discrimination loss 15–20 /	13	2.9
Discrimination loss 25–50 /	5	1.1
Discrimination loss over 60 /	1	0.2
Total	451	100.0

Discrimination was 100 per cent for 405 ears (89.8 per cent) A marked discrimination loss, over 60 per cent, was established in only one ear Discrimination loss was established in two cases among the 79 ears for which the pure tone audiogram was normal. It was 10 per cent in one and 25 in the other case.

Fowler's test was possible in 63 cases (24.6 per cent) Table 13 gives the results.

TABLE 13 — *Fowler test*

Loudness recruitment	Number f cases
Complete	39
Partial	19
None	5
Total	63

The recruitment phenomenon was negative in only five cases (7.9 per cent)

The adaptation test was performed on 478 ears (93.4 per cent) The results are given in Table 14

TABLE 14 — *Adaptation test*

Hearing threshold shift	Number f ears
0–15 dB	298
20–50 dB	127
over 50 dB	53
T tal	478

Adaptation of over 30 dB was established in 55 ears (11 1 per cent) In three ears it was over 50 dB although the pure tone audiogram was normal. Adaptation of over 30 dB was established also in an ear with poor conductive hearing loss. Hence, 396 ears with sensori-neural or combined hardness of hearing included 49 with adaptation of more than 30 dB (12.4 per cent)

E Results of the General Neuro-otological Tests

Romberg s test was performed on all the patients. It was positive in 22 cases (8 6 per cent)

The stepping test was succesful for 190 patients (74.2 per cent) Table 15 shows the rotation and the angle of rotation.

TABLE 15 — *Rotation and angle of rotation in th stepping test*

Rotation	Number of patients	Per cent
Does not rotate	82	43.2
Rotation under 90 deg	97	51.0
Rotation 91—180 deg	9	4 7
Rotation over 180 deg	2	1.1
Total	190	100 0

Eighty two (43.2 per cent) f the patients who performed the stepping test did not rotate at all. The angle of rotation was under 90 deg with 97 patients (51.0 per cent) Only two patients rotated more than 180 deg.

Past-pointing was tested for ll the patients. The test was positive in 11 cases (4.3 per cent).

F Vestibular Special Studies

1 SPONTANEOUS AND POSTURAL NYSTAGMUS

Spontaneous nystagmus was demonstrated electronystagmographically in 12 cases (4 7 per cent) The vari tion in amplitude and frequency was completely irregular in all the cases. Spontaneous nystagmus was very slight in four cases for which only

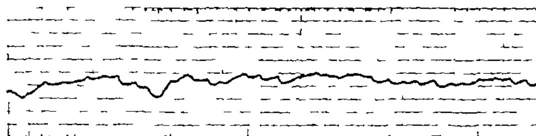


FIG 5 – *Spontaneous nystagmus.*

a few isolated nystagmus strokes were recorded. One case involved latent spontaneous nystagmus. Fig 5 illustrates a case of spontaneous nystagmus. In only two cases was spontaneous nystagmus distinctly visible on inspection.

Postural nystagmus was observable at inspection in *56 cases (21.9 per cent)*

2. RESULTS OF THE CALORIC TEST

Cold water calorisation in both ears was performed on 251 patients.

The duration was measurable in 315 ears (62.7 per cent) The time of termination of the reaction could be established accurately in 906 cases, but caused some difficulty in the other cases. However even in these cases it was possible to determine with fair accuracy Because of occasional indistinctness of the curve due to a technical disturbance, duration was impossible to measure in some cases although calorisation produced a definite reaction.

The duration values are given in Table 16

TABLE 16 – *Duration during water calorisation at 30°C*

Duration	Number of cases	Per cent
under 60 sec.	18	5.7
60–99 sec.	126	40.0
100–130 sec.	122	38.7
over 130 sec.	49	15.6
Total	315	100.0

Durations of under 60 sec. totalled 18 (5.7 per cent) and durations longer than 130 sec. totalled 49 (15.6 per cent)

Fig 6 shows the distribution of durations in cold water calorisation in normal cases and ears with lesions. Definite differences in duration were established between the two groups. Furthermore the durations varied greatly within the traumatic group Duration of over 150 sec., but also of less than 50 sec., were established. On the other hand, the duration range was generally from 100 to 160 sec. in the cases of normal reaction.

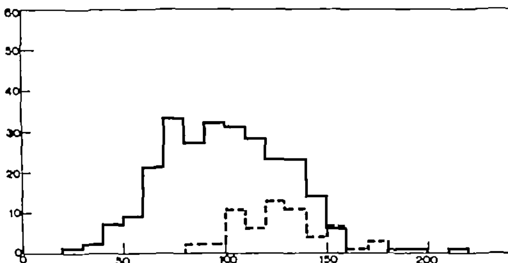


FIG 6. — *Distribution of duration in cold water calorisation. Normal reactions totalled 60 — broken line. Cases with lesions totalled 255 — solid line. The number of cases is the ordinate and the duration in seconds the abscissa.*

It was possible to determine the maximum intensity in 245 ears. The mean was 7.42 deg./sec. The material included a great many cases of severe hypoexcitability which made it impossible to determine the maximum intensity.

The results of the determination of the latent period are given in Table 17.

TABLE 17 — *Latency*

Time of onset of reaction	Number of cases	Per cent
Reaction begins during calorisation (under 30 sec.)	66	21.0
Reaction begins 31–40 sec. after start of calorisation	145	46.0
Reaction begins 41–50 sec. after start of calorisation	75	23.8
Reaction begins 51–60 sec. after start of calorisation	27	8.6
Reaction begins over 60 sec. after start of calorisation	2	0.6
Total	315	100.0

There were only 66 cases (21.0 per cent) in which the reaction started during calorisation.

Different types of electronystagmography curve were obtained from the cold water calorisation test. They are shown in Table 18.

The reaction was regular in both amplitude and frequency in the regular curve type. In the irregular curve type the variation in both amplitude and frequency was completely irregular. This curve type was recorded in a total of 220 cases (43.8 per cent) and predominated in the material. The group of irregular reactions included 10 cases of dysrhythmia. Cases with no reaction at all to water calorisation at 30°C were also fairly numerous, 181 in all (36.0 per cent).

TABLE 18 – *Types of electronystamograph curve*

Type of curve	Number of cases	Per cent
Regular	76	15.1
No reaction	181	36.0
Irregular	220	43.8
Frequency fluctuations	7	1.4
Amplitude fluctuations	18	3.6
Total	502	100.0

Fig. 7 shows a regular and irregular type of curve and a part of the electronystagmograph curve for a case of dysrhythmia.

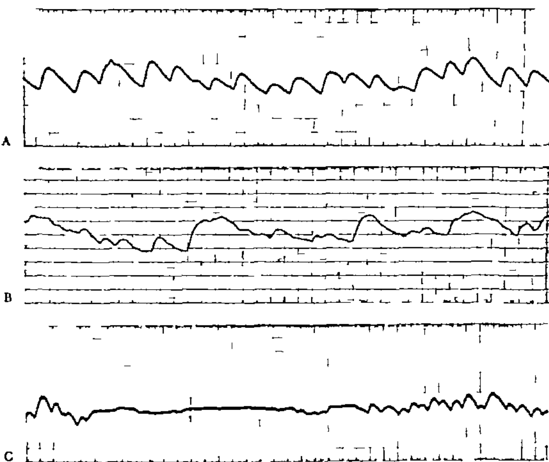


FIG. 7. – *A. Regular reaction. B. Irregular reaction. C. dysrhythmia.*

Hot water caloristation after cold water calorisation was performed on 233 patients.

The duration could be measured for 230 ears (49.4 per cent) only. Table 19 shows the duration values.

TABLE 19 — *Duration during water calorisation at 44°C*

Duration	Number of cases	Per cent
under 60 sec.	40	17.4
60— 99 sec.	95	41.3
100—150 sec.	77	33.5
over 150 sec.	18	7.8
Total	230	100.0

Durations of under 60 sec. numbered 40 (17.4 per cent). Durations longer than 150 sec. totalled 18 (7.8 per cent). Durations of under 60 sec. were thus distinctly more numerous in hot water than in cold water calorisation (17.4 per cent > 5.7 per cent), and those over 150 sec. fewer than in cold water calorisation (7.8 per cent < 15.6 per cent). In addition, duration was measurable much less often in hot than in cold water calorisation (49.4 per cent < 62.7 per cent) because the reaction was much more infrequent to hot water calorisation.

Maximum intensity was determinable in 165 ears, mean 6.19 deg./sec. Cases of severe hypoexcitability were even more numerous in the hot water than in the cold water test, and it was not possible to determine the maximum intensity for these cases.

Table 20 gives standard calculations for duration and maximum intensity during cold and hot water calorisation and during cold water calorisation in normal and traumatised cases.

TABLE 20 — *Standard calculations*

	Number	Mean	Standard deviation	Standard error
Mean duration, cold water calorisation	166	109.1	27.8	2.16
Mean duration, hot water calorisation	166	91.3	30.8	2.39
Mean duration, cold water calorisation, normal reaction cases	60	124.5	21.6	2.80
Mean duration, cold water calorisation, cases with lesions	255	96.6	29.7	1.86
Mean maximum intensity cold water calorisation	245	7.12	3.67	0.24
Mean maximum intensity hot water calorisation	165	6.19	3.04	0.238
Mean maximum intensity, cold water calorisation, normal reaction cases	60	10.07	3.94	0.509
Mean maximum intensity, cold water calorisation, cases with lesions	185	6.56	3.15	0.229

Both cold and hot water calorisation was performed on these 166 patients.

The mean duration in cold water calorisation was 109.1 sec. and in hot water calorisation 91.5 sec. In calculating the mean only the cases tested with both cold and hot water calorisation were considered. The difference 109.1 > 91.5 is statistically significant. The mean duration for cold water calorisation in the cases with a normal reaction was 124.5 sec., and in the cases with lesions 96.6 sec. The difference, 124.5 > 96.6 is statistically significant.

The mean maximum intensity in cold water calorisation was 7.42 deg./sec. and in hot water calorisation 6.19 deg./sec. The difference 7.42 > 6.19 is fairly small but nevertheless statistically significant. The mean maximum intensity in cold water calorisation in the normal reaction group was 10.07 deg./sec. and in the injured group 6.56 deg./sec. The difference 10.07 > 6.56 is statistically significant.

Directional preponderance was demonstrated in 47 patients, i.e. 18.4 per cent of the total material. However it could only be tested in 255 patients on whom both cold and hot water calorisation was performed.

Table 21 shows the result of the vestibular study of 251 patients.

TABLE 21 — *Result of the vestibular study*

		Number of patients	Per cent
Bilateral symmetrical finding	normal reaction	24	9.6
	hypoexcitability	83	33.1
	no response	69	27.5
	canal paresis	28	11.1
	directional preponderance	47	18.7
Total		251	100.0

A bilateral-symmetrical vestibular finding was made for 176 patients (70.2 per cent). The vestibular finding was normal for both ears in only 24 patients (9.6 per cent). The deviation of the vestibular reaction of either ear from normal was clear in all 83 cases of bilateral-symmetrical hypoexcitability. Twenty eight cases (11.1 per cent) involved canal paresis, that is relative vestibular hypofunction of one ear compared with the contralateral ear.

Air calorisation was performed on both ears of five patients.

The duration could be measured for three ears. It was 70, 90 and 110 sec. In two cases duration began during calorisation and in one case only 75 sec. after the beginning of calorisation. Accurate determination of the termination of the reaction was possible in all three cases.

The electronystagmography curve was irregular in three ears and no reaction was elicited in another seven. The result of the vestibular study of these five patients was as follows: three patients had no reaction in either ear, vestibular hypoexcitability was established in both ears of one patient, and one patient had canal paresis.

The forms of vestibular disturbances suggesti e of *cent al les on* are given in Table 22.

TABLE 22 – *Central vestibular lesion*

	Number f patients
Spontaneous nystagmus	12
Directional preponderance	17
ENG-dysrhythmia	6 (10)
Bilateral normal hearing and bil teral estibular lesion	17
Total	82

Four of the 10 cases f dysrhytmia belonged to the directional preponderance group

Indications of central vestibular les on were seen n 82 patients (32.0 per cent) of the 256 given a vestibular examination.

VII OTHER REASONS EXTRA TO TRAUMA FOR HEARING AND VESTIBULAR DEFECTS

The principal reason for the hearing and vestibular defects in the present series was a wound resulting in brain injury However there may have been other contributory factors, especially as it was quite a long time between the injury and the present study The case reports were searched for clues as to possible other reasons, but the finding is based in part on the patient's own account. The information is very inadequate, but it helps to som extent to explain the etiology of the current neuro-otological status of the patients.

A Factors Affecting Hearing Before the Wound

No objective information was available on the patients hearing at the beginning of the war Eight patients said that their hearing was definitely impaired at the time the war broke out.

According to their own statements, eight patients had had a purulent discharge from the ears before they were wounded. One of them had undergone ear surgery for chronic otitis media.

Ten patients had suffered a minor skull injury during the war before the wound that resulted in brain damage These wounds had all been closed and many of them had been caused by a bullet or a splinter

Sixteen patients reported that they had been so close to an explosion that tinnitus or loss of hearing ensued. No hearing tests were performed for most of these cases.

B Injuries after the Wound

Fifteen patients sustained a skull injury resulting in the loss of consciousness. Complete loss of hearing in one ear was the consequence for two of them Seven of these injuries occurred during the war, eight later

One patient sustained ear damage from a detonation as a result of which he became markedly hard of hearing in one ear

One patient had worked for several years in conditions of intense noise and this had probably contributed to the hearing loss.

C Diseases

Nine patients had meningitis or a brain abscess after being wounded

One patient had otosclerosis. Stapedectomy was performed on both ears.

One patient had suffered an acute loss of hearing in one ear for an unknown reason. His hearing improved somewhat later but h was still very hard of hearing in one ear

There was one case of congenital harelip and cl ft palate This patient had bilateral chronic adhesive otitis.

A wartime history of lues was involved in seven cases. The disease h d been properly managed in the early stage in each of these cases and there was no information on possible sequelae.

D Ototoxic Drugs

Seven patients had obviously received streptomycin for pleurisy or pulmonary tuberculosis. Nothing was known, however about possible drug induced inner ear damage nor whether the drug h d been streptomycin sulphate or dihydrostreptomycin. Almost all th patients had been gi en an ample measure of various drugs fo long periods as treatment for the symptoms caused by the brain injury Many f them had taken analgesics regularly Various ataracts had been employed as sedatives. Patients suffering from epilepsy had regularly taken hydantoin derivatives, barbiturates and Mysoline. The patients were also under the influence f ordinary medication during the neuro-otological examination. It was not considered possible to abandon medication because of the risk of deterioration f their disease

VIII CORRELATION BETWEEN THE NEURO-OTOLOGICAL STATUS AND LOCALISATION OF THE INJURY

A Unilateral deafness was probably the result of trauma in 23 cases. The localisation of the injuries in these cases was distributed as shown in Table 23

TABLE 23 – *Localisation of the injury in th cases of unilateral deafness*

Localisation f injury	Number of cases	Total of injuries
Temporal	13	48
Frontal	4	61
Occipital	1	22
Parietal	1	6ɔ
Facial	1	23
Concussion-contusion injuries impossible to localise more precisely	3	62
Total	23	

Most of the cases of unilateral deafness were caused by an injury to the temporal region. In seven of the 13 cases with unilateral deafness caused by a temporal injury the wound had affected the immediate aural region.

B A bilaterally normal pure tone audiogram was recorded in 28 cases. Table 24 reveals the localisation of the injury in these cases.

TABLE 24 – *Localisation of the injury in the cases with normal hearing*

Localisation of injury	Number of cases	T tal of injuries
Parietal	7	63
Frontal	6	61
Temporal	5	48
Facial	3	23
Occipital	2	22
Concussion-contusion injuries impossibl to localise more precisely	5	62
Total	28	

Injuries of different types were represented fairly evenly in the patients with normal hearing

C. In 72 cases there was no vestibular reaction from eithe ea The localisation of the injuries in these cases is presented in Table 25.

TABLE 25 – *Localisation of the injury in th cases with no estibular reaction from either ear*

Localisation f injury	Number f cases	Total f injuries
Frontal	19	61
Parietal	10	65
Facial		25
Temporal	6	48
Occipital	6	2_
Concussion-contusion injuries impossible to localise more precisely	21	62
Total	72	

Concussion-contusion and frontal injuries were in the majority of the cases with severe bilateral vestibular dam ge.

D The vestibular reaction was normal bilaterally in 24 cases. The localisation of the injury in these cases is given in T ble 26.

TABLE 26 – *Localisation f the injury in the cases with intact estibular function*

Localisation f injury	Number of cases	T tal f injuries
Parietal	9	65
Occipital	4	22
Frontal	1	61
Facial	1	25
Temporal	0	48
Concussion-contusion injuries impossible to localise more precisely	9	62
Total	4	

There were no temporal injuries in the cases with intact estibular function.

E. Aphasia was established in 17 patients t an examination immediately after they were wounded. *Fowler* test was performed on three patients with aphasia. The recruitment phenomenon was positiv in all of them. The discrimination score was 100 per cent for 16 patients. One patient had unilateral 45 per cent discrimination loss and deafness in th other ear Not single case of discrepancy between the pure tone udiogram and threshold fo speech discimination was established.

IX DISCUSSION

Hearing defects

According to the literature, the incidence of hearing defects caused by skull injuries varies. However many investigators have encountered them in about a half of the patients sustaining a skull trauma (*Schuknecht* and *Davison* 1956 *Ey* 1966) The studies have generally been concerned with injuries arising in peacetime. The present investigation established impaired hearing in 89 1 per cent of the patients an average of 23 years after they were wounded. In the age group to which the patients of the present series belong changes of such magnitude that they are of essential importance for the evaluation of the hearing examination results are not caused by pure presbyacusis (*Leuti* 1949)

Skull injuries usually lead to sensori neural or combined hearing defects. Purely conductive hearing loss is rare This was established by e.g. *Piquet* and *Decroix* (1954) Maximum hearing loss is usually observed in high tones (*Schuknecht* and *Davison* 1956 *Proctor et al.* 1956, *Lumio* and *Aho* 1962) Sensori neural hearing defects predominated also in the present material. Ears with sensori-neural hearing defects accounted for 82.4 per cent of all ears with a loss of hearing. In contrast, only 2.9 per cent of the ears had conductive hearing defects. The maximum hearing loss was in the higher tones in the majority of cases. The commonest type of audiogram was the descending type of curve, 75 per cent of the ears. The descent began at 2000 Hz in 46 per cent of the ears. Ears with sensori neural hearing defects and those with combined hearing impairment were taken into consideration when determining the curve types. However there was again a rise in the higher frequencies in 58 per cent of the declining types of curves.

There was not a single completely deaf patient in the material, but 25 (9.8 per cent) patients had unilateral deafness. Deafness was probably the sequela of the wartime injury in 23 cases. Only 28 (10.9 per cent) patients had a normal pure tone audiogram. Although there were relatively few patients with normal hearing, the material included quite a number with a good mean hearing threshold. The mean was 25 dB or better for roughly 60 per cent of the hearing ears. This was due to a great extent to the fact that the frequencies 500, 1000 and 2000 were taken into consideration in calculating the mean and that more pronounced impairment of hearing occurs only after 2000 Hz. Thus, it was also possible to determine the speech thresholds in many cases, and it was 45 dB or better in 73 4 per cent of these ears.

Fowler test is regarded as the most reliable method of differentiating between cochlear and retrocochlear lesion. *Dix Hallpike* and *Hood* (1948) considered a positive recruitment phenomenon to suggest damage to Corti's organ According to current research, this is the case in most instances. Discrepancy between the pure tone audio-

gram and threshold for speech discrimination, and poor speech discrimina... regarded as indicative of retrocochlear lesion (*Harbert* and *Young* 1964 *Elcher* ... 1966, *Fowler* and *Altmann* 1966 *Benitez et al.* 1966) But opinions differ as to the significance of adaptation. Most workers consider that strong adaptation occurs more often in retrocochlear than in end organ lesions (*Palva* 1964 *Benitez et al.* 1964 In general, too great diagnostic significance should not be attached to adaptation Moderate adaptation, in particular may occur in many kinds of hearing defects It was possible to perform *Fowler's* test on 63 (24 6 per cent) of the patients in the present material. The recruitment phenomenon was negative in no more than 9 per cent of the cases. Discrepancy between the pure tone audiogram and threshold for speech discrimination was established in only 5 per cent (24 cases out of 48?) The discrimination score could be determined in 92.6 per cent of the hearing ears. No discrimination loss at all was established in 89.8 per cent. A discrimination loss of over 50 per cent was established in only 0.2 per cent. It was possible to perform the adaptation test on 478 ears (93.4 per cent). Adaptation was over 30 dB in 11 1 per cent. Although their pure tone audiogram was normal, three cases displayed adaptation of over 30 dB similarly adaptation of over 30 dB was established in an ear with conductive hearing loss. *Palva* (1964) also reported that strong adaptation can sometimes occur in ears with normal hearing.

Most workers regard traumatisation of an end organ, possibly in association with damage to the auditory nerve as the commonest reason for impairment of hearing after a skull injury Advocates of a central etiology are fewer (*Zange* 1915 *Kecht* 1965). The present study suggests that the hearing impairment established in brain injured ex-servicemen was mostly due to damage to an end organ. Examination of the 17 patients who developed aphasia after being wounded strengthen this opinion. No signs of a central hearing defect were demonstrated in these patients either although some of the cases must have involved injury to the temporal lobe None of the patients with aphasia revealed discrepancy between the pure tone audiogram and threshold for speech discrimination, and discrimination loss was established in one patient only Moreover the recruitment phenomenon was positive in all the cases in which it was possible to perform *Fowler's* test.

Vestibular damage

The incidence of vestibular damage caused by skull traumas is also known to be very high, in some cases even higher than that of hearing defects (*Lumio* and *Aho* 1962). Vestibular damage was established in 90 6 per cent of the present series, which is little different from the incidence of hearing defects.

Disturbances of equilibrium were present in 71.5 per cent of the patients. Fifty per cent of the material reported by *Gurdjian* and *Webster* (1958) complained of vertigo. The corresponding percentage in *Ey's* material was 61

Spontaneous nystagmus was established electronystagmographically in 4 7 per cent of the material. The nystagmus was very faint and irregular in all the cases. *Pirodda* and *Cenacchi* (1964) observed that spontaneous nystagmus demonstrated long time

reason for the complete hearing loss in one ear In addition, a reason other than traumatisation was found to have caused the severe hearing loss in at least two cases. Otosclerosis was diagnosed bilaterally in one case Another patient had chronic adhesive otitis bilaterally which was possibly due to congenital harelip and cleft palate. These factors account for the conductive hearing loss in at least two cases. No objective information was available on ear diseases suffered before the war or on the hearing of the patients when the war started. However eight patients told that their hearing was distinctly impaired at the outbreak of the war

The drug therapy given to the patients, above all epilepsy drugs and sedatives, may have influenced the vestibular function in particular The patients in fact continued with their normal medication at the time of examination. *Aschan et al.* (1956) reported that postural nystagmus was sometimes demonstrable in patients under sedation during psychiatric treatment. *Mehra* (1964) noted that anticonvulsive medication has an inhibiting effect on duration, total of strokes and amplitude in nystagmus.

Effect of the localisation of the injury on hearing and vestibular defects

Blows to the occipital region very readily cause a sensori-neural hearing loss, according to *Schuknecht* and *Davison* (1956) However temporal injuries were the cause of the most severe hearing defects in the material described by *Caliceti* and di *Fede* (1964) which comprised only closed skull-brain injuries. In the present material, too, it was temporal injuries that caused the greatest number of cases of unilateral deafness in 15 out of 23 cases. However these temporal wounds included seven injuries to the immediate region of the ear Traumas affecting the immediate aural system totalled eight, and there was thus only one case in which the ear escaped a complete hearing loss. However there were also patients with normal hearing who had suffered temporal injuries, and no d finite differences between various types of injury were observable. The cases with normal vestibular function included no temporal injuries at all. It would seem, then, that a temporal trauma always leads to vestibular damage Severe bilateral vestibular disturbances were caused chiefly by traumas of concussion-contusion type.

X SUMMARY

The purpose of the investigation was to throw light on the incidence and nature of hearing defects and vestibular lesions in brain injured ex-servicemen. Additionally the proportion of peripheral and central hearing and estibular lesions in the material and possible correlations between the neuro-otological finding and the localisation f the injury were studied.

The material consisted of 256 patients wounded in the Finnish wars of 1939—1940 and 1941—1944 who had been found on neurological examination to have a brain injury as the sequela of the wound.

All the patients were given a pure tone and speech audiometric examination. To establish loudness recruitment, *Fowler's* test was performed on the cases in which a distinct difference was stated in the hearing ability of the ears at the hearing threshold level. Adaptation was measured at the threshold level by observing the shift in the hearing threshold during three minutes. The most important part of the vestibular study was the caloric test which was recorded electronystagmographically. Calorisation was done with water of 30°C and 44 C and in five cases with air of room temperature.

The audiologic examinations revealed that 89.1 per cent of the patients had impaired hearing. Unilateral deafness was established in 9.8 per cent. There was not *single case of complete deafness in the series. A sensori-neural hearing impairment* was the commonest hearing defect in the material. The commonest of the audiogram types was the descending curve, and th depression occurred most ften at 2000 Hz. *Fowler's* test, the adaptation test, speech discrimination and the examination of the possible discrepancy between pure tone audiogram and speech threshold led to the conclusion that the majority of the hearing defects were of end organ origin.

Vestibular damage was established in 90.6 per cent f the patients. Th majority of the injuries were bilateral-symmetrical (~~50.0 per cent~~) Symptoms suggestive of a central vestibular lesion were encountered in 52 per cent of the material. Spontaneous nystagmus, directional preponderance, electronystagmography dysrhythmia, and association of bilaterally normal hearing with bilateral vestibular lesion were taken as symptoms indicative f a central lesion. The patients had spontaneous nystagmus or directional preponderance of very long duration. Contrary to the hearing defects which appeared to be mostly peripheral, numerous symptoms suggestive of central vestibular damage were encountered.

Although the main cause of the present hearing defects and estibular disturbances was wound resulting in a brain injury there were other contributory factors. This especially as fairly long time had elapsed from the original injury It appeared .g. *that the complete hearing loss in one ear had occurred post-traumatically for another* reason in at least two cases. The chemotherapy administered t the p tients, above all

epilepsy drugs and sedatives, may have affected first and foremost the vestibular status.

A temporal injury was the commonest reason for unilateral deafness. However in such cases the trauma had often affected the immediate aural region. The hearing of some of the patients remained normal in spite of the temporal injury But it always appeared to cause vestibular damage.

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S U P P L E M^E N T U

INFLUENCE OF SOME STREPTOMYCES ANTIBIOTICS ON THE INNER EAR OF THE GUINEA PIG

Electrophysiological and histological study

F OSTYN and J TYBERGHEIN

ACTA OTO-LARYNGOLOGICA NARVAVÄGEN 16, STOCKHOLM NO

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SUPPLEMENTUM 234

From the Department of Otorhinolaryngology
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CONTENTS

INTRODUCTION

The mechanism of inner ear intoxication by ototoxic antibiotics has not yet been fully elucidated, although numerous recent electrophysiological and histochemical studies and research by electron microscope have been carried out about this problem.

Keidel and his team studied the influence of streptomycin on the adaptation of the cells of Corti. Engström's remarkable research by means of electron microscopy has been able to locate very early lesions in the sensory cells. Kohonen examined the intoxication pattern of the sensory cells with phase contrast microscopy. With his histochemical technique Müsebeck was able to demonstrate alterations in the stria vascularis before histological and measurable functional disturbances are found in the inner ear. Rauch and Voldrich have shown a prolonged presence of the otoxic product in the endolymph and the perilymph.

Nevertheless the results obtained are rather divergent. Not only is there an important difference in sensitivity of the various species of animals to the ototoxic antibiotics, but also in each species the individual sensitivity is very different.

Personally we experimented on guinea pigs because these animals lend themselves easily to the purpose of combined electrophysiological and histological studies. Moreover it is rather simple to work with relatively large series of guinea pigs. In order to obtain constant results most of our electrophysiological measurements (J. T.) were carried out on series of 15 ears. We (F. O.) performed careful histological controls on at least 5 ears in each series of animals.

Purpose of the experiment

1. Finding the location of the lesions caused by ototoxic antibiotics in the whole of the inner ear and the chronology of their appearance.
2. Comparison of the toxicity of the antibiotics used.
3. Testing of certain drugs with a view to a reduction of the ototoxicity of certain antibiotics.
4. Establishing any possible relation between a decrease in cochlear microphonics and an accurate location of the lesions.

In order to have at our disposal a constant test material, we chose the homogeneous breed of guinea pigs from the Animal Virous Diseases Research Institute Pirbright, England. The composition of the fodder has been kept constant with a supplement of fresh vegetables and hay. The temperature and percentage of moisture of the air in the cages were equally

kept constant. Thus we suppose that the laboratory animals show less variation in their characteristics and their reactions to the administered products.

We experimented with kanamycin, streptomycin, viomycin, capreomycin and neomycin.

1 EXPERIMENTAL TECHNIQUE

I Functional tests

A The Preyer reflex

Before the first injection of the antibiotic to each series of animals, the auditory function of the animals was examined by means of a high frequency whistle as used with the training of dogs The guinea pigs that did not show a good Preyer reflex were excluded from the experiment While the antibiotic was being administered and also three weeks after stopping the injections the Preyer reflex was checked in the same way to detect any possible deterioration in hearing

The results of the Preyer reflex are not mentioned in this paper since the examination of the auditory function with this technique averred to be inexact and seemed to have only an indicative value

B The vestibular tests

Three weeks after stopping antibiotic injections, the vestibular rotation tests were performed on all the animals. The guinea pig was placed on an electrically powered rotating disc in a long narrow box. The rump of the animal was thus completely fixed and only the head could move slightly This allowed us to examine the deviation during and after the rotation. The eyes were not covered The rotations were done in both clockwise and anticlockwise directions at the rate of ten revolutions in twenty seconds. With normal experimental animals the average duration of the post rotatory nystagmus after ten clockwise or anticlockwise revolutions in twenty seconds is eleven seconds. The results of the rotation tests with the various series of animals are mentioned each time in the description of the histological examination of the vestibular system.

Since streptomycin is specially toxic to the vestibular system, the animals treated with this antibiotic were carefully examined for vestibular disturbances every week. The following findings were noted the presence of ataxia (disturbed walking and attitudes, purposeless spontaneous turning) the presence or absence of the labyrinthine righting reflex, the deviation during and after rotation and the duration of the postrotatory nystagmus with clockwise and anticlockwise turning

C. The cochlear microphonics

We measured the influence of certain ototoxic antibiotics on the cochlear microphonics with the guinea pigs. The registering technique used has been

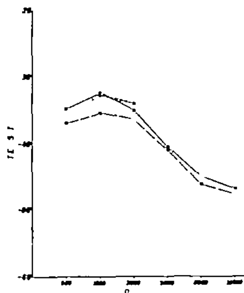


FIG 1 Comparison between the three isotonic saline gro ps (15 ears in each group) at 100 db stimulation intensity —— group used as co trol in the kanamycin experiments; — — group sed as control in the streptomyci xperiments, - - - group used as co trol in the neomyci experiments.

published in detail earlier (*Acta Oto-laryng* (Stockh) 1962, Suppl *171*) Under an intraperitoneal 10% Veterinary nembutal anaesthesia (1/10 cc per 200 g body weight) a metallic electrode was placed in the round window niche after the bulla has been opened, and the neutral electrode was fastened on the glandula submandibularis

The preparation was placed in an electrically and acoustically shielded room with a loudspeaker at 75 cm from the ear drum We measured the microphonics with 80 90 and 100 db stimulation intensity at 500 1000 2000 5000 8000 and 10 000 c/s. We express the value of the microphonics in db loss to 10 mv consequently −20 db = 1 mv −30 db = 315 μv −40 db = 100 μv −50 db = 31.5 μv and −60 db = 10 μv

The whole of our experimental technique is based on the comparison be tween the cochlear microphonics of normal control animals that were in jected with isotonic saline and those of series of guinea pigs that were given certain doses of ototoxic antibiotics. That our results are reliable is shown by Fig 1 which depicts the reactions of our three groups of fifteen normal ears these curves are completely superimposable

II Histology

A Histological technique

Imn l t ly aft r recording the cochlear microphonics and under the same intraperitoneal ana thesia a sufficient number of experimental animal n ea h g p were intravitally fixed, so that we always had at our

1 I lo-l y g

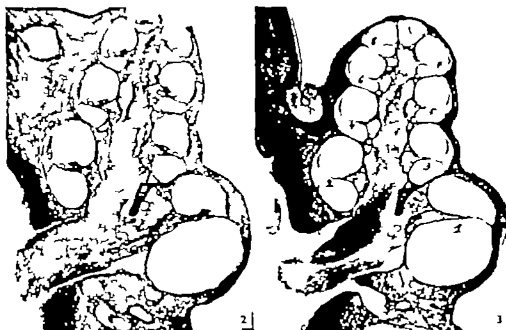


Fig. 2. Normal midmodiolar section through the cochlea of guinea pig. Celloidine preparation, 16 μ.

Fig. 3. Midmodiolar section through the cochlea of guinea pig with the numbering of the organs of Corti.



Fig. 4. Normal organ of Corti with surrounding structures. Second turn of the cochlea of a guinea pig.

FIG. 5. Schematic drawing of a cross section of the second turn. (Davis, *Neural Mechanisms of the Auditory and Vestibular Systems*, p. 22.)

disposal some twenty well fixed ears. Ears with inflammatory lesions in the region of the bulla or badly fixed preparations were systematically excluded. We call attention to the fact that we examined mainly those ears which according to the reading of the microphonics, were most or at least strongly impaired.

The animals were fixed *intravitally with the solution of Heidenhain Suza* according to the technique of Schuknecht (1953). Both temporal bones were extracted and opened. The stapes were taken out or luxated and a small hole was drilled in the apex of the cochlea. The temporal bones were then fixed, decalcified, neutralized, cleaned, dehydrated and embedded

TABLE 1. *Arrangement of the two types of external hair cells (E.H.C.) in relation to the tunnel (T) and to the internal hair cell (I.H.C.) on the four cochlear turns*

Turn	I.H.C.	T	E.H.C.
4th	I.H.C.	T	A. B. B.
	I.H.C.	T	A. B. B.
	I.H.C.	T	A. A. B.
	I.H.C.	T	A. A. A.

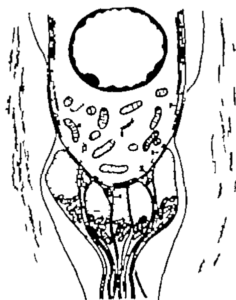
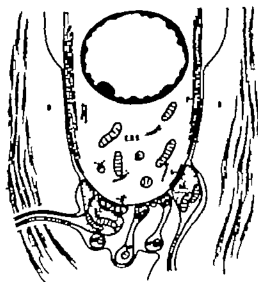


Fig. 6.



F g. 7

F 6. Schematic drawing of Type A hair cell from the third cochlear turn. (Smith, C. A. & Sjöstrand, F. S., *J Ultrastructure Research*, *4* 1961.) *A* accessory membrane *B*, synaptic bar *C* region of vesicle concentration; *D*, Deiters' cell; *EHC*, external hair cell; *M* mitochondrion, *N* nucleus, *1* type 1 nerve ending; *NE*, type 2 nerv ending; *P* vesicular peripheral membranes, *SV* synaptic vesicles.

F 7 Schematic drawing of Type B hair cell. (Smith, C. A. & Sjöstrand, F. S., *J Ultrastructure Research*, *4* 1961.) *A* accessory membrane *B*, synaptic bar *D* Deiters cell, *EHC* external hair cell, *M* mitochondrio *N* nucleus, *1* type 1 nerve ending; *2* type 2 nerve ending; *NE* 2a, type 2 nerve ending; *P* vesicular peripheral membranes, *SP* spiral nerve fiber *SV* synaptic vesicles.

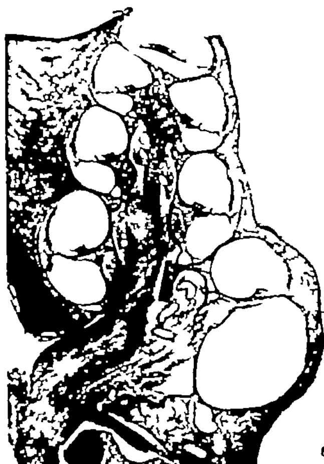
in increasing concentrations of celloidine The celloidine cubes were cut up in the longitudinal axis of the cochlea and parallel to the plane of the N Acusticus. The gauge of the histological slice was 10 μ and each fifth slice was kept for histological examination. The vestibular system was

TABLE 2 *Diagram of the arrangement of the two types of E.H.C on the four cochlear turns*

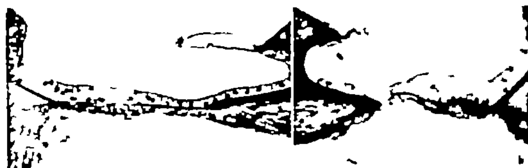
Th rem l l g types re printed i italic commented pon i Figs. 10 nd 11 This di gram learly illustrates the earlier damage of the type A cell.

Guinea pig treated with daily dose f eomycl 100 mg/kg body weight for 20 days. G.P No. 21 L.

Turn	E.H.C.	T	T	E.H.C.
Fourth	(8) *B* *B* A.	T	T	A. *B* *B* [illegible]
Third	(6) *B* *B* A.	T	T	A *B* *B* (5)
Second	(4) *B* A. A.	T	T	A. A. B (3)
F rst	(2) A. A. A.	T	T	A. A. A. (1)



Fi 8 Midmodiolar section through the cochlea of a guinea pig, treated with a daily dose of neomycin 100 mg/kg body weight for 30 days. G.P Neo, 21 L. The organs of Corti of the four turns are enlarged and commented upon in the following figures. The destruction in the basal ganglia I easily seen.



I a. rg rt (b) Orga of Corti I Cross section through the basal t rn. C inea pig treat th of neomycin 100 mg/kg body weight for 30 d s. C P Neo, 21 I Total dest I Corti, which is replaced by typical cells. The cells of Boettcher are the onl the E.H C. (A. \. \.) are consequently destroyed.

I I lo-I J



Fig. 10. (a) Organ of Corti 4. (b) Organ of Corti 3. Cross section through the second turn. Guinea pig treated with a daily dose of neomycin 100 mg/kg body weight for 30 days. G.P. Neo, 21 L. Organ of Corti 3: The I.H.C. is intact. The tunnel shape remains without tunnel cells. The E.H.C. (A. A. B.) are destroyed. Organ of Corti 4: Normal I.H.C. and tunnel. The most lateral of the E.H.C. is intact, *B*. A. A., the remaining type of E.H.C. being printed in italic.

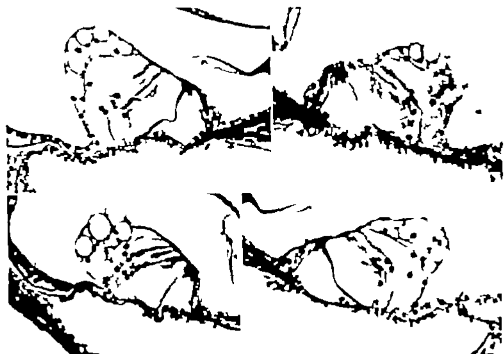


Fig. 11. (a) Organ of Corti 6. (b) Organ of Corti 5. (c) Organ of Corti 8. (d) Organ of Corti 7. Cross section through the third and fourth turn. Guinea pig treated with a daily dose of neomycin 100 mg/kg body weight for 30 days. G.P. Neo, 21 L. The four organs of Corti present the same picture: The I.H.C. and tunnel are normal. The two lateral E.H.C. are intact. The most medial one is destroyed. *B B* A. (Corti 6–8) A. *B B* (Corti 5–7), the remaining types of E.H.C. being printed in italic. Looking at the E.H.C. on the micrographs, one must remember that the histological section, made on 10 µ, may contain two rows of E.H.C. One may easily count three or four E.H.C. but not six.



FIG. 12. Ea ly damage of the most central of th L.H.C. (type A cell) as seen by the ordinary light microscopy The volume of the cell I reduced and the nucleu I displaced in pical direc tion toward the ticle of the hair cell. N te the loose hair cell in the tu nel. Fourth t rn. Guinea pig treated with treptomycin, total dose f 15 g/kg body weight. G.I 5 /8 R.

Fi 13. The three F.H.C. are affected. The cells re mall and the nuclei are displaced t ward the cuticl Basal t rn Guinea pig treated with a daily dose f k namy in 200 mg/kg body w ght d of Nialamide 15 mg/kg body weight for 30 days. G P N /10 I

further cut up in the same direction and each fifth slice was also kept 11 pecimens were stained by Van Gieson s method

ter hi t l g cal examination a graphic reconstruction of the cochlea wa rried t c rding to Guild (1921) and Schuknecht (1953) Any pos i lesion I I in the organ of Corti the stria vascularis the gang lion ale an l t l us were also represented graphically according to Schukn ht (18





F 14. The nuclei f th I.H.C. are absent and those of the cell of Deiters are displaced towards the lamina spiralis. Loose hair cell in Nuel' space. Basal turn. Guinea pig treated with daily dose of kanamycin 200 mg kg body weight and f Nialamide 8 mg/kg body weight for 30 days. G.P N_0/11 R.

F g. 15. Not the absence of the nuclei of the three E.H.C. Basal turn. Guinea pig treated with daily dose of kanamyci 200 mg/kg body weight and of Nialamide 8 mg/kg body weight for 30 days. C P N_0/1 L.

B Histological results

Some of the antibiotics used have a stronger effect on the peripheral vestibular system than on the cochlea, while others have the reverse Some are very toxic, others less so But histologically the impairment always follows the same pattern, so that the same description counts for all the antibiotics used.

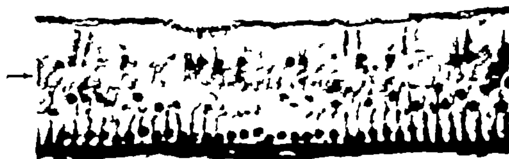


Fig. 16 Longitudinal section through the t nel. On imilar section f normal guine pig, w see row f nuclei f th I H C. abo the pillars. Here w only f d some scattered nuclei (arrow). First nd second t rn. Guinea pig treated with dail dose f ka m ci dipantothe- nat 250 mg kg body w ight for 7 days. C P P S I

I *Histology and histopathology of the cochlea*

In order to elucidate the hi to path lo gy we first describe so me normal histo logical pictures. Fig 2 shows a midmodi lar section through the cochlea In Fig 3 the different organs of Corti are numbered fro m 0 to 9 to permit ea y location of the lesi ns. Fig 4 represents a normal secti n of the organ of Corti with the surrounding tructures The schematic repr esen tation with the names is to be f und in Fig 5

(a) *Lesions in the organ of Corti* The effect of all ot it oxic antibiotics on the sensory cells seem to f llow the same pattern The lesi ns start in the basal coil and devel p gradually in the direction of the apical coil

The external hair cells are impaired fir t in each coil, starting at the most central and spreading to the two lateral Thi confirms the results f Engstro m & K honen (1965) This finding applies also to the basal coil but it i much more obvious in the higher coils. Thi intoxication pattern is certainly connected with the various types of external hair cells de- scribed by C. A Smith and F S Sj ostrand (1961) C. A Smith di tingui hes a type A cell (Fig 6) and a type B cell (Fig 7) according to the difference in innervati n

The arrangement of the vario u types in the different coils is represented in Tal le 1 The type A cell i imp aired more quickly than the type B cell This i clearly illu trated by the Figs. 8 9 10 and 11

Hawkin & Eng tr m (1963) stated that one of the earliest changes cau ed b y t t xic antibiotics is a disturbance of the orderly W pattern of the tereocilia f th uter h r cell in the basal coil while Kohonen (1965) found the n hanges in t outer hair cell to be the first pathologic phen men

Thi can t r let rmin d b y an of light microscopy and with the plane f secti n l n With ligh r copy the first symptom observed



F o. 17 Destruction f the supporting cells (Deiters, Hensen) of the organ of Corti. Basal turn. Guinea pig treated with daily dose of neomycin 100 mg/kg body weight for 30 days. G.P Neo$_3$/20 L.

F o. 18. Destruction of all supporting lement of the organ f Corti. Absence f the inner hai cell and of som ucld of the limbus. The cells of Boettcher are i tact. Basal turn. Guinea pig treated with daily dose of neomycin 100 mg/kg body weight for 30 days. C P Neo$_3$/21 L.

are a reduction in volume and a shrinking of the intoxicated cell The cell becomes small and bulbous the nucleus moves towards the cuticle of the cell The latter eventually disappears or the cell breaks loose and wanders in the tunnel or in the ductu cochlearis. (Figs. 12, 13, 14 15 and 16)

After the outer cells have disappeared, the supp rting cells collapse the cell of Deiters, Hensen and Claudius are affected and disappear and the tunnel tructure collapses slowly (Figs. 1 and 18)

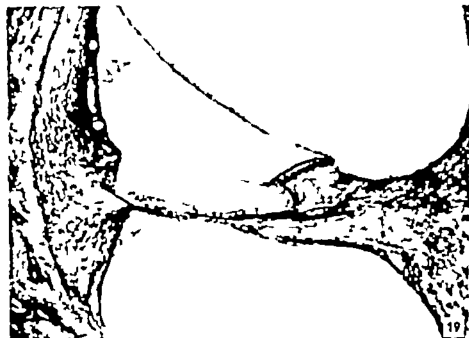


Fig. 19 The organ of Corti is completely destroyed and replaced by undifferentiated cuboid epithelium. The cells of Boetcher are still present. The nuclei of the limbus are absent and the number of nerve fibres in the lamina spiralis ossea is reduced. Basal turn. Guinea pig treated with a daily dose of neomycin 100 mg/kg body weight for 30 days. (P. Neo/21.)

The internal hair cell is very resistant but disappears as well following prolonged intoxication. Finally the whole organ of Corti is replaced by undifferentiated cuboid epithelium (Fig. 19).

The cells of Boetcher, characteristic for the basal coil, remain unimpaired, even in this last stage.

The total collapse of the organ of Corti after disappearance of the sensory cells is in most cases more obvious from two millimetres onwards than in the first two millimetres. The intoxication of the sensory cells has been graphically expressed as 100 % when the internal as well as the three external hair cells were absent, as 75 % when only the three external hair cells were absent and as 0 % when all the cells were still present (Figs. 41 and 40).

(b) *Lesions in the stria vascularis.* C. A. Smith studied the structure of the stria vascularis with the electron microscope. She described the following cell types:

1. Epithelial cells.

Marginal cells, described as chromophil cells (Von Fieandt & Saxen 1930). The cytoplasm contains large mitochondria, a large concentration of granules and many membrane structures. The basal cell membrane is composed of foldings and fingerlike projections.

Lighter ... chromophobe cells. These cells are found between the



FIG. 20. Cross section through the stria vascularis of normal guinea pig. The different cells, described by C. A. Smith, are easily recognizable. Basal turn. G.P 8 R.

FIG. 21. Picture of obvious injury to the stria vascularis. The regular design of the chromophil cells is destroyed and the cells are fused, without recognizable nucleus or borders. The number of chromophil and chromophobe cells is reduced and the width of the stria vascularis is decreased. Basal turn. Guinea pig treated with a daily dose of kanamycin monopantothenat 250 mg/kg body weight for 20 days. G.P P 14 L.

marginal and the basal cells. They contain only a scattering of ergastoplasma.

2. Basal cells: double layer of thin flat cells.

The stria vascularis is widest at the basal coil and becomes narrower towards the apex. The normal stria vascularis is represented in Fig. 20. With light microscopy it is difficult to trace the first lesions in the stria vascularis. In most cases we find the chromophil cells hyperchromatic probably caused by a disturbance of the mitochondria. The cell outlines become thus less distinct (Fig. 21). The stria vascularis becomes narrower and the number of chromophil cells diminishes. Eventually the stria becomes like a thin tape often hyperchromatic without definite structure and often with rents (Fig. 22).

Though it is difficult to study the stria vascularis with light microscopy the impairment seemed nevertheless more severe from two millimetres onwards than within the first two millimetres of the basal coil. As we have no clear insight into the remaining function of the stria vascularis which, according to the microscopic examination, seems fully damaged, this injury



F o. 22. T t l destruction of the proper cell nd their composition in th tri scalaris. Basal t rn. Guinea pig treated with daily lose of neomycl 10 l mg kg body w ight for 30 da s. C l Neo 71 L.

has been graphically represented as 50%. The lower degree of impairment is represented as 25% (Fig 40)

(c) *Lesions of the neuron and of the ganglion spirale* It is clear that the lesion cf the ganglion spirale are secondary After the de truction of the external sensory cell and especially after the comp lete collapse of every architecture cf the organ of Corti we see a decrease cf the radial running nerve fil res in the tunnel and of the nerve fibres in the lamina spiralis ossea The ganglic n cells degenerate and disappear (Figs. 23 and 24) There is a decrea e cf the nerve fibres in the Nervus Acu ticus in the corre pond ing zone

The reduction of the ganglion cells was estimated at 75 when strongly reduced at 0 or 25% when not so strongly reduced acc rding to the degree f imp airment (Fig 70)

(d) *Lesi n f the limbus* When the organ of C rti is strongly affected the nuclei f the c ll ll ap pear fr m the limbus. Thi impairment l mo t important the l l corresponding to the zone cf 2 to 6 mm in the basal coil (I g ?

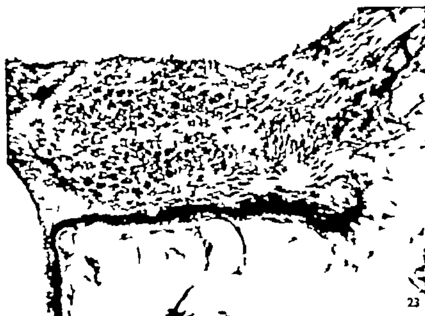




Fig. 23. Cross section through the ganglion spirale of normal guinea pig. Basal turn. G.P. 8 R.

Fig. 24. Cross section through the ganglion spirale of guinea pig, treated with daily dose of neomycin 100 mg/kg body weight for 30 days. G.P. Neo./21 L. Basal turn. Note the loss of ganglion cells, estimated at 75 %.

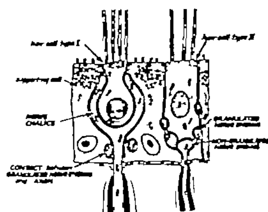




F1 . 3. Cros section through th limbus of normal guinea pig. Basal t rn. C I 8 B.

Fig. 26. Cros section through the limbu of guinea pig, treated with dail dose f neomycel 100 mg/kg bod ight f 30 days. C I Neo, 21 L. Basal turn. Not th bsence of the nuclei f the limb d the d r se f th density f the nerv fibres i the lamina spiralis ossea.

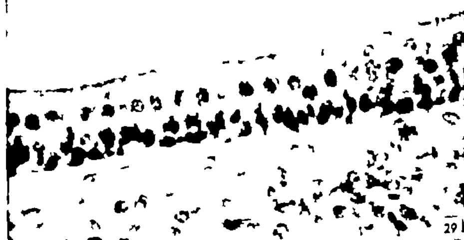
A la lo-larg g S 11)



F o. 27 Schematic drawing of the vestib lar sensory epithelium as it appears in cat, guinea pig, and rat. (Wersäll. *Neural Mechanisms of the Auditory nd Vestibular Systems*, p 232.)



F 28. Cros section through the crista ampullaris f normal guinea pig. G.P 8 R.



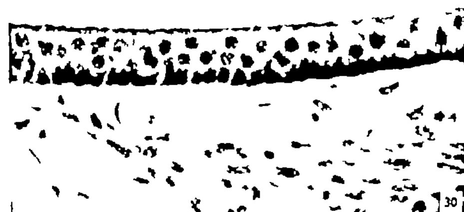


Fig. 29 Longitudinal section through part of the crest of the cri ta ampullaris. Normal guinea pig. C 1 11 R.

Fig. 30 Section through normal macula sacculi Guinea pig treated with capreomycin, total dose of 15 g kg body weight C 1 C/3 R

The complete disappearance of the nuclei has been represented diagrammatically as 100% intoxication when some nuclei remained this has been plotted as 50% intoxication (Fig 79)

(e) *Lesions in the surroundings* When the organ of Corti is very strongly impaired we sometimes see a decrease of the number of nuclei in the bony wall of the cochlea especially in the wall that lies freely in the bulla consequently corresponding to the organ of Corti 1 and 2



Fig. 31. Section through the macula utriculi of normal guinea pig. G.P 1 L.



Fig. 32. Early damage to the vestibular sensory cells by an ototoxic antibiotic, as seen with the light microscope The supra-nuclear protoplasm protrudes towards the endolymph (arrow). Section through the macula utriculi of guinea pig, treated with daily dose of kanamycin-dipa totbenal 250 mg/kg body weight for 20 days. G.P P_3/10 R.





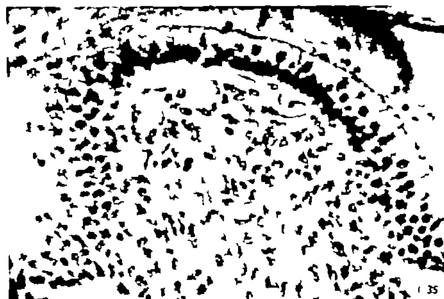
Fig. 33. Longitudinal section through the crista ampullaris of a guinea pig, treated with a daily dose of kanamycin monopantothenate 50 mg/kg body weight for 20 days. G 1 I 5 H. Note the ejection of one nucleus and of the protoplasm of two cells.

Fig. 34. Section through the macula utriculi of a guinea pig, treated with daily dose of kanamycin dipantothenate 250 mg/kg body weight for 20 days. C I I 10 H. Two cells are ejected with their nucleus and their protoplasm.

II. *Histology and histopathology of the peripheral vestibular system*

The macula sacculi and utriculi and the cristae ampullares of the three semicircular duct were studied by means of the light microscope.

Wersall J. (1954) gives a detailed description of the vestibular sensory cells (Fig. 27).





F 35. Cross section through the crista ampullaris of guinea pig, treated with streptomycin, total dose f 15 g/kg body weight. G.P S_2/4 R. Several nuclei are jected. The loss of sensory cells t the crest of the crist is already ob ious.

F 36. Longitudinal section through the crest of the crista ampullaris f guinea pig, treated with treptomycin, total dose of 15 g/kg body weight G P S_2/4 R. N t the marked loss f sensory cells and some flatness f the sensory epithelium.

The normal vestibular structures as seen with the light microscope ar represented in Figs. 28, 29 30 and 31

Duvall & Werall (1964) studied the effects of streptomycin on the u per ear sensory epithelia in guinea pigs by means of the electron micros p The most severe damage was found in the vestibular sensory cell L with degeneration of and myelin figure formation in the mitoch Later swelling appeared in the sensory hairs with deformati n o



Fi 37 Cros section through th crist mpollari of guinea pig, treated with trept mycin, tot l dose f l g kg body weight C I S_3 l II. Marked los of sensory cell more or les through out the sensory epithelium f th cri ta.

surface and finally disappearance of the sensory hairs, swelling of the cell surface often with rupture and ejection of cells and cell debris into the endolymph

With light microscop y nly the later stages of the intoxication could be observed First we see a bulging of sup ranuclear protoplasm towards the endolymph (Fig 32) Afterwards the whol content of the cell with the nucleus is ejected (Figs 33 and 34) The thickness of the sensory epithelium diminishes, the supporting cells disap pear and here and there only a few sensory cells are left (Figs 35 36 37 and 38) With further intoxication all sensory epithelium disappears (Figs 39 and 40)

The lesion are most severe in the macula of the utriculus the cristae present a stronger degree of intoxication on the crest The macula of the sacculus is only slightly if at all affected by these antibiotics in the doses we administered

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F g. 38. Section through the macula triculi f a guinea pig treated with treptomycin, tot l dose f 15 g/kg body weight C I S /6 R. Marked loss of sensory cells.

F g. 39 Section through the macula triculi f guinea pig treated with streptomycin, total dose of 15 g/kg body weight G.P $S_3/6$ R. Severe damage t the macula triculi

F 40. Section through the macula triculi of guinea pig treated with streptomycin, total dose of 15 g/kg bod weight C I $S_3/6$ R. T tal destruction of th macula utriculi.


38


39


40

2 KANAMYCIN

Three groups of guinea pigs were given five days a week subcutaneous injections of kanamycin sulphate. We administered a daily dose of 100 mg/kg body weight to group K_3 for 30 days (total dose 3 g/kg body weight). Group K_5 was given a daily dose of 250 mg/kg body weight for 20 days (total dose 5 g/kg body weight) and group K_6 was given a daily dose of 200 mg/kg body weight for 30 days (total dose 6 g/kg body weight).

Three other groups of guinea pigs were injected subcutaneously five days a week with 200 mg/kg body weight of kanamycin sulphate a day for 30 days (total dose 6 g/kg body weight). These groups were called $N_{1.5}$, N_8 and N_{15}. In these groups each injection with antibiotics was accompanied by a subcutaneous injection of respectively 1.5 mg/kg body weight, 8 mg/kg body weight and 15 mg/kg body weight of nialamide.[1] Kanamycin and Nialamide were injected with different syringes and needles at two different places.

Finally three last groups of guinea pigs called P_1, P_2 and P_3 were injected subcutaneously five days a week for 20 days with a daily dose of 250 mg/kg body weight of a given kanamycin pantothenate (total dose 5 g/kg body weight). Group P_1 was given kanamycin monopantothenate, group P_2 was given kanamycin dipantothenate and group P_3 kanamycin tripantothenate. A control group was given a daily subcutaneous injection of 0.25 cc of isotonic saline.

The general condition of all the animals in this test remained good except in the groups K_6 and N_{15} where the animals lost lots of hair; there was however no mortality (Table 3).

Three weeks after the last injection the cochlear microphonics of 15 ears in each group were recorded, and about 10 animals were intravitally fixed immediately after this registration.

I. Cochlear microphonics

In the following diagrams (Figs. 41, 42 and 43) we have compared first the groups K_3, K_5 and K_6 with the group that received the isotonic saline, then the groups $N_{1.5}$, N_8 and N_{15} with group K_6, and finally the three kanamycin pantothenate groups with group K_5.

We notice that a daily dose of 100 mg/kg body weight of kanamycin

[1] Niamid®, Pfizer.

TABLE 3 *Mortality rate and weight evolution during the test*

Animal group	No. of animals alive		Average weight	
	On first injection	At moment of recording	On first injection, g	At moment of recording, g
K_3 (kanamycin sulphat 3 g/kg)	20	20	239.7	582.0
K_5 (kanamycin sulphat 5 g/kg)	20	20	239.2	491.0
K_6 (kanamycin sulphat 6 g/kg)	20	20	237.2	576.0
N (K_6 + 1.5 mg/kg Nialamide)	20	18	238.7	572.4
N (K_6 + 8 mg/kg Nialamide)	20	20	243.0	537.0
N_{15} (K_6 + 15 mg/kg Nialamide)	20	20	236.5	543.[illegible]
P (kanamycin monopantothenat 5 g/kg)	20	20	240.2	517.7
P (kanamycin dipantothenat 5 g/kg)	20	20	217.0	467.0
P (kanamycin tripantothenat 5 g/kg)	20	20	239.0	530.2
Isotonic saline	20	20	252.5	583.0

TABLE 4 *Average reaction of the 15 ears of the isotonic saline group*

The value of ... is given in parentheses. This group was used as control.

Frequency c/s	80 db	90 db	100 db
500	40.20 (±8.71)	37.13 (±9.43)	34.60 (±8.88)
1,000	39.70 (±8.80)	35.53 (±8.94)	32.20 (±6.78)
2,000	37.00 (±7.00)	34.93 (±6.24)	34.86 (±8.24)
5,000	45.06 (±6.32)	42.00 (±6.55)	40.26 (±8.00)
8,000	52.66 (±4.35)	48.66 (±5.65)	44.86 (±5.47)
10,000	52.06 (±4.47)	49.00 (±7.00)	46.60 (±5.09)

TABLE 5 *Average reaction of the 15 ears of the K_3 group*

The value of ... is given in parentheses.

Frequency c/s	80 db	90 db	100 db
500	39.26 (±8.47)	36.66 (±5.65)	34.93 (±5.09)
1,000	41.10 (±6.08)	36.80 (±5.65)	33.40 (±4.88)
2,000	39.63 (±6.16)	37.84 (±5.38)	36.86 (±4.60)
5,000	46.60 (±6.55)	44.80 (±6.16)	42.73 (±5.47)
8,000	54.33 (±4.12)	50.66 (±5.28)	47.33 (±5.09)
10,000	54.46 (±4.21)	51.53 (±5.29)	48.80 (±5.00)

ulphate administered for 30 days (total dose 3 g/kg body weight) hardly produced any drop in the microphonics (Fig 41) There is no statistically significant difference between group K_3 and the one that received the isotonic saline In 1962 we did find a statistically significant drop of the microphonics with the same intoxication schedule The explanation for this seeming contradiction is very probably to be found in the fact that we now

TABLE 6 *Average reaction of the 15 ears of the K_1 group*

Th[illegible] of [illegible] gi[illegible]en [illegible] parentheses.

Frequency c	80 db	90 db	100 db
[illegible]	45.53 (±3.[illegible])	4[illegible] (±[illegible])	4[illegible] (±3.53)
1[illegible]	49.33 (±[illegible])	4[illegible] (±[illegible])	45.53 (±[illegible])
2[illegible]	4[illegible].66 (±[illegible])	4[illegible] (±6.[illegible])	4[illegible] (±6.33)
3[illegible]	51.66 (±3[illegible])	53.[illegible] (±[illegible])	[illegible] (±3.3)
8[illegible]	[illegible].86 (±2.64)	[illegible] (±[illegible])	5[illegible].13 (±3.[illegible])
10[illegible]	[illegible] (±[illegible])	3[illegible] (±3.1)	[illegible] (±3.1[illegible])

TABLE 7 *Average reaction of the 15 ears of the K group*

The [illegible] of [illegible] gi[illegible]en in parentheses.

Frequency c	80 db	90 db	100 db
[illegible]	32.[illegible] (±3.[illegible])	31.33 (±6.32)	[illegible] (±[illegible])
1	33.33 (±3.3)	[illegible] (±[illegible])	[illegible] (±[illegible])
[illegible]	31.[illegible] (±6.[illegible])	32.[illegible] (±6.[illegible])	[illegible].93 (±6.32)
3[illegible]	3[illegible] (±3.[illegible])	[illegible] (±3.[illegible])	[illegible] (±[illegible])
8[illegible]	[illegible] (±1.[illegible])	[illegible].13 (±2)	3[illegible].33 (±2.[illegible])
10[illegible]	[illegible] (±1.[illegible])	[illegible] (±1.73)	[illegible] (±2)

TABLE 8 *Average reaction of the 15 ears of the N_{13} group*

The [illegible] of [illegible] gi[illegible]en in pare[illegible]theses.

Frequency	80 db	90 db	100 db
[illegible]	31.26 (±3.[illegible])	[illegible] ([illegible])	[illegible].33 (±3.[illegible])
1[illegible]	31.68 (±3.[illegible])	31.33 (±6.[illegible])	3[illegible] (±[illegible])
2[illegible]	36.[illegible] (±6.16)	[illegible] (±6.[illegible])	33.[illegible] (±3.63)
3[illegible]	3[illegible] (±3.[illegible]6)	3[illegible].60 (±3.[illegible])	3[illegible] (±3.31)
8[illegible]	3[illegible] (±1.73)	3[illegible] (±1.73)	3[illegible].93 (±2)
10[illegible]	3[illegible] (±1.41)	3[illegible].33 (±1.41)	[illegible] (±1.41)

TABLE 9 *Average reaction of the 13 ears of the N group*

The [illegible] of [illegible] gi[illegible] [illegible] pare[illegible]theses.

Frequency	80 db	90 db	100 db
[illegible]	33.[illegible] (±3.[illegible])	31.[illegible] (±3.[illegible])	[illegible].33 (±3.[illegible])
1	31.[illegible] (±4.[illegible])	3[illegible] (±6.[illegible])	33.13 (±[illegible])
2[illegible]	31.46 (±[illegible])	3[illegible] (±3.01)	33.33 (±3.[illegible])
3[illegible]	3[illegible] (±[illegible])	3[illegible] (±3.[illegible])	[illegible] (±3.[illegible])
8	[illegible] (±1.[illegible])	[illegible].33 (±[illegible])	[illegible] (±2.[illegible])
1[illegible]	[illegible]13 (±1.41)	3[illegible] (±1.73)	3[illegible].86 (±2.[illegible])

TABLE 10 *Average reaction of the 15 ears of the \ group*

The valu f is gi en in parentheses.

Frequency c/s	80 db	90 db	100 db
500	52.86 (±5.00)	51.20 (±5.47)	49.53 (±5.91)
1,000	52.53 (±5.83)	51.60 (+7.07)	50.66 (±8.06)
2,000	53.80 (+8.00)	52.86 (±7.68)	52.13 (±7.07)
5,000	56.26 (±5.83)	55.73 (±6.08)	55.20 (±5.19)
8,000	58.00 (±3.31)	57 73 (±4.35)	57.53 (±3.46)
10,000	58 13 (±3.46)	58.13 (±3.74)	58.33 (±2.44)

TABLE 11 *Average reaction of the 15 ears of the P group*

The l of is give in pare theses.

Frequency /s	80 db	90 db	100 db
500	40.66 (±8.85)	38.73 (±7 41)	39.40 (±7.61)
1,000	40.80 (±8.40)	37 40 (±7 48)	36.60 (±8.18)
2,000	40.40 (±8.18)	39.53 (±8.24)	40.33 (±7.54)
5,000	47 06 (±6.65)	45 46 (±7.28)	45.53 (±7.34)
8,000	52.00 (±5.38)	49.86 (±8.00)	48.66 (±6.83)
10,000	53.53 (±4.24)	51 46 (±5.38)	50.26 (±5.74)

TABLE 12 *Average reaction of the 15 ears of the P group*

The alue f I given in pare theses.

Frequency c/s	80 db	90 db	100 db
500	43.06 (± 9 64)	43.00 (±10.67)	40.80 (±10.14)
1,000	45.60 (± 8.83)	42.40 (±11.04)	40.93 (±11.87)
2,000	44.93 (±10.39)	44.00 (±11.18)	44.30 (±10 19)
5,000	51.06 (± 7.00)	50.20 (± 8.24)	49 13 (± 8.79)
8,000	56.40 (± 4.12)	53.40 (± 8.00)	51.93 (± 7 14)
10,000	53.93 (± 3.87)	53.93 (± 5.19)	53.00 (± 5.81)

TABLE 13 *Average reaction of the 15 ears of the P group*

The value f is given in pare theses.

Frequency /s	80 db	90 db	100 db
500	45.60 (±6.40)	43.20 (±8.55)	41.33 (±5.63)
1,000	45.40 (±6.16)	42.06 (± .34)	40.00 (±7 14)
2,000	45.26 (±6.63)	43.60 (±6.78)	43.73 (±6.00)
5,000	51.60 (±5.54)	50.13 (±5.56)	49.66 (±5.19)
8,000	53.33 (±3.80)	53.46 (±4.35)	52.06 (±4.35)
10,000	56.00 (±3.31)	51.86 (±3.60)	54.00 (±3.87)

TABLE 14 *Value of F (1.29) where the reaction is statistically better in the normal animals (isotonic saline group) than in the K_4 group on the 18 examined points*

Test on 15 ...rs. It lk l pe: $P < 0.01$

Frequency c/s	80 db	90 db	100 db
500	20.95	33.51	29.35
1,000	27.50	35.03	45.25
2,000	45.25	49.50	61.69
5,000	46.01	47.56	73.46
8,000	27.50	36.71	61.29
10,000	33.33	46.73	65.40

TABLE 15 *Value of F (1.29) where the reaction is statistically better in the normal animals (isotonic saline group) than in the K_3 group on the 18 examined points*

Test on 15 ...rs. It ll type: $P < 0.01$

Frequency	80 db	90 db	100 db
500	9.34	12.15	16.13
1,000	13.43	16.10	26.82
2,000	23.29	33.07	37.6
5,000	21.37	29.67	30.61
8,000	14.71	23.41	43.37
10,000	19.40	26.33	41.50

TABLE 16 *Value of F (1.29) where the reaction is statistically better in the normal animals (isotonic saline group) than in the I_1 group on the 18 examined points*

Test on 15 ears. R ma type $0.01 < P < 0.05$

Frequency c/s	80 db	90 db	100 db
500	—	—	—
1,000	—	—	—
2,000	—	—	4.07
5,000	—	—	1.60
8,000	—	—	—
10,000	—	—	—

have at our disposal more purified preparations and genetically pure breeds of animals, which live under constant experimental conditions. The important drop in microphonics that we find in group K_4 (Fig 41 Tables (and 14) we find again entirely in groups N , N and N_{13} (Fig 42) Consequently the Nialamide does not protect the microphonics of the guinea pig against the harmful influence of kanamycin sulphate

TABLE 17 *Value of F (1.28) where the reaction is statistically better in the normal animals (isotonic saline group) than in the P_2 group on the 18 examined points*

Test 15 ears. Italic type $P < 0.01$ Roman type $0.01 < P < 0.05$.

Frequency /s	80 db	90 db	100 db
500	—	—	—
1 000	—	—	6.09
2,000	6.01	7.52	*9.12*
5,000	7.29	*9.43*	*10.4*
8,000	—	4.87	*9.39*
10,000	6.29	6. 8	*10.13*

TABLE 18 *Value of F (1.28) where the reaction is statistically better in the normal animals (isotonic saline group) than in the P group on the 18 examined points*

Test on 15 ears Italic type $P < 0.01$ Roman type $0.01 < P < 0.05$

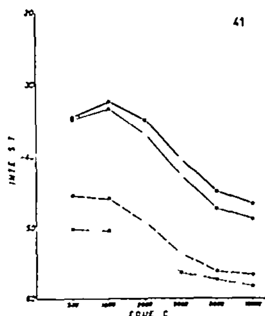
Frequency /s	80 db	90 db	100 db
500	—	—	6.02
1,000	4.71	4.76	*9.30*
2,000	*10.79*	*13.83*	*14.64*
5,000	*9.05*	*13.33*	*21.03*
8,000	—	6.72	*14.55*
10,000	7.50	*12.94*	*30.15*

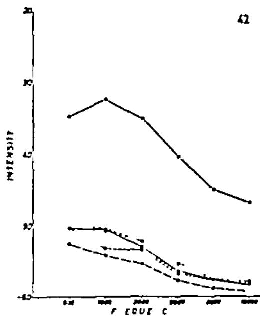
TABLE 10 *Value of F (1.28) where the reaction is statistically better in the P than in the K group on the 18 examined points*

Test on 15 ears. Italic type $P < 0.01$

Frequency /s	80 db	90 db	100 db
500	*11.66*	*11.69*	*8.46*
1,000	*11.93*	*14.15*	*10.33*
2,000	*10.61*	*11.60*	*11.37*
5 000	*12.16*	*12.94*	*11.67*
8,000	*14.44*	*14.60*	*14.34*
10,000	*12.06*	*12.16*	*13.74*

The situation is completely different with the kanamycin pantothenates in comparison with the kanamycin sulphate the kanamycin monopantothenate is almost non toxic to the microphonics of the guinea pig (Fig 43 Only at a few points (Table 16) is there a statistically significant intoxication in the group P whereas there is a very outstanding statistically significant difference between groups P_1 and K at the 18 points





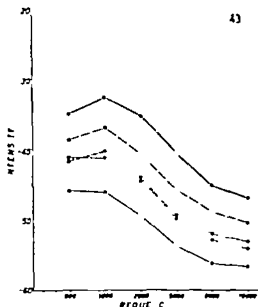


FIG. 41 Comparison between the I ears of the isotonic saline group and those f th K (kanamycin lphat 3 g/kg), K (kanamyci sulphat 5 g/kg) nd K₆ (kanamycin sulphat 6 g/kg) groups t 100 db timulation intensity —— isotonic saline group; —— K group; — — K group; - K₆ group.

F G. 42. Comparison between the 15 ears of th isotonic salin group nd those of the K (kanamycin sulphat 6 g/kg), N (K nd 1 5 mg/kg day Nialamid), N (K₆ and 8 mg/kg/day Nialamide) nd N (K nd 15 mg/kg/da Nialamid) groups t 100 db timulation I tensity ——, isotonic saline group, — K group; — — — N group; - N group, — — N₁₅ group.

FIG. 43. Comparison between the 15 ears of the isotonic saline group and those f the K (kanamycin ulphat 5 g kg), P (k nam yci monopantothenat 5 g/kg), P (kanamycin dipa tothenat 5 g/kg) and P (kanamycin tripantothenat 5 g/kg) groups t 100 db timulation I tensit —— isotonic saline group — K group; — — — P group; P group; — — — P group.

examined (Table 19) This reduced toxicity is only partly found again with the kanamycin dipantothenate and the kanamycin tripantothenate (Tables 17 and 18) These results confirm our conclusions of 1962 completely

II Histological results

Group K

Guinea pigs treated with a daily dose of 100 mg/kg body weight of kanamycin sulphate for 30 days.

The group K_3 was not examined histologically in these experiments any more since in a previous histological research, of which the results remained unpublished, no lesions were registered with guinea pigs of the same genetic breed, treated with kanamycin sulphate following the same schedule Moreover the microphonics did not drop in this group

Group K_4

Guinea pigs treated with a daily dose of 200 mg/kg body weight of kanamycin sulphate for 30 days.

In this group six ears have been examined histologically These were taken at random, since all 15 ears showed a drop in their microphonics

A *Cochlea*

With all the animals the outer hair cells have disappeared for various distances from the beginning of the basal coil The diagram of the missing sensory cells in the six ears we examined is represented in Fig 44

So we see that the outer hair cells have disappeared completely from the basal coil up to an average distance of 6.53 mm, and that they are fully present again at an average distance of 8.2 mm in the animals we examined. Table 20

Table 20 *Distances are given over which all the external hair cells have disappeared and the distances to the point where all the external hair cells are present again in the ears examined*

The average values are calculated.

Guinea pigs, Group K_4	Absence of the three E.H.C. (mm)	Presence of the three E.H.C. (mm)
1 L	10	10
5 L	8.3	15.2
6 R	9.4	9.4
9 L	2.7	3.1
11 L	6.8	8.7
12 L	2	2.8
Average distance	6.53	8.2

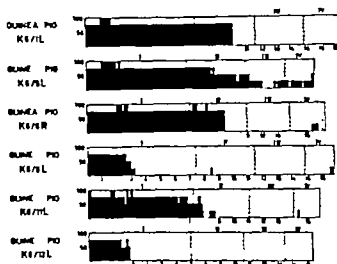


Fig. 44. Diagram of the destruction of the cochlear hair cells, plotted in black, in six ears of guinea pigs treated with a daily dose of 200 mg/kg body weight of kanamycin sulphate for 30 days.

The stria vascularis is markedly impaired in the area where the external hair cells have completely disappeared. The impairment is less pronounced towards the apex, but covers nevertheless almost the same distance as the *impairment of the external hair cells.*

The ganglion spirale corresponding to the first half of the basal coil is slightly affected. The ganglion cells may have dropped to 25 % in animals with heavy impairment of the hearing, but in most cases some cells have a swollen nucleus or a nucleus in lysis.

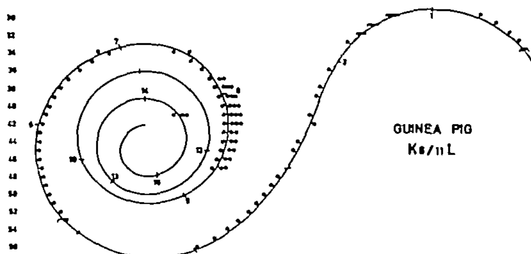


Fig. 45. Diagram of the cochlear spiral of guinea pig K_s/11 L with the remaining sensory cells. For the sake of clearness of the curve the cells still present have been plotted only on the distance of impairment. The figures indicate the distance in mm, starting from the basal coil (p. The hatched areas represent complete destruction of the organ of Corti with replacement by undifferentiated cuboid epithelium.

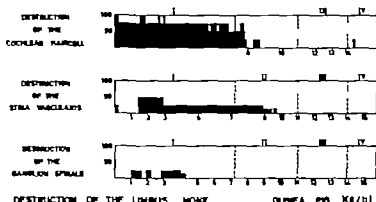


Fig. 46. Diagrams of the destructions of the sensory cells, of the stria vascularis and of the ganglion cells in guinea pig K₈/11 L. The destructions are represented as 0/50/100 % as explained in Section 1. The Roman numerals indicate the coils of the cochlea starting at the basal coil as coil 1. The Arabian figures indicate the distance of the cochlea in mm starting from the basal coil up.

The limbus and the surroundings are completely normal.

The cochlear diagrams of guinea pig K_8/11 L are fully represented in Figs. 45 and 46.

B. *Vestibular system*

The average duration of the postrotatory nystagmus in 20 animals, stimulated by 10 revolutions in 20 sec, clockwise and anticlockwise was 9.7 sec and 10.2 sec respectively.

No histological anomalies were established in the vestibular system.

Group N_{13}

Guinea pigs treated with a daily dose of 200 mg/kg body weight of kanamycin sulphate and 1.6 mg/kg body weight of Nialamide for 30 days.

This group was not examined histologically.

Group N

Guinea pigs treated with a daily dose of 200 mg/kg body weight of kanamycin sulphate and 8 mg/kg body weight of Nialamide for 30 days.

Of this series five ears also taken at random were examined since all ears showed a decrease of the microphonics.

A. *Cochlea*

The sensory cells, especially the external hair cells, have disappeared for [illegible] distance from the beginning of the basal coil as represented in Fig. 47. The average distance over which all the external hair cells are m[illegible]ng is 4.75 mm in these ears examined, whereas the average distance

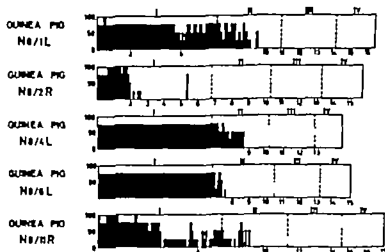


FIG. 47 Diagram of the destruction of th cochlear hai cells, plotted in black in fi ears of guinea pigs treated with daily dose f 200 mg/kg body weight of kanamyci lphat nd f 8 mg/kg body weight of Nialamide for 30 days.

to the point where all the external hair cells are present, is 7 44 mm (Table 21)

In all these animals the severity of damage to the stria vascularis is proportionate to the extent of destruction of the external hair cells

The ganglion spirale is only very little affected The impairment consists of a lysis of the ganglion cells, especially in the ganglion corresponding to the organ of Corti No 1

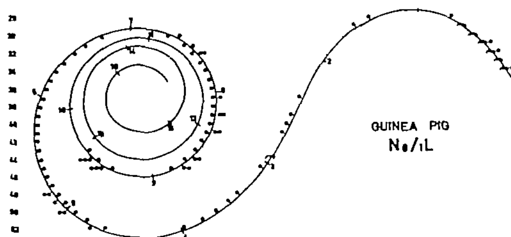
The limbus and the surroundings are normal

The cochlear diagrams of the guinea pig N /1 I are fully represented in Figs. 48 and 49

TABLE 21 *Distances are given over which all the external hair cells have disappeared and the distances to the point where all the external hair cells are present again in the ears examined*

The erage l es re calcul ted

G inea pigs, Group N.	Absence of the three I H C. (mm)	Presence f th three I H.C. (mm)
1 L	1 8	9.8
2 R	1 9	2.6
4 I	7.2	8.5
6 I	7	7.5
11 R	3	9
A er ge distance	4.78	7 44



F a. 48 Diagram of the cochlear spiral of the guinea pig N_2/1 L with the remaining sensory cells. For the sake of clearness of the curve the cells still present have been plotted only on the distance of the impairment.

B *Vestibular system*

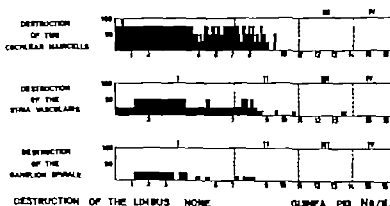
The average duration of the postrotatory nystagmus in 17 animals stimulated by 10 revolutions in 20 sec clockwise and anticlockwise was 12.4 sec and 13 sec respectively

Histologically all the vestibular structures are normal

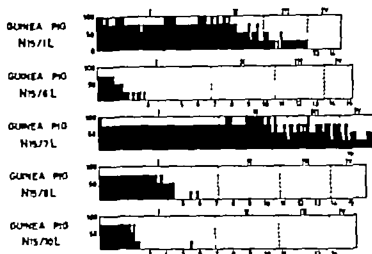
Group N_1

Guinea pigs treated with a daily dose of 200 mg/kg body weight of kanamycin sulphate and 15 mg/kg body weight of Nialamide for 30 days.

From this group five ears also taken at random were examined, since all ears showed a decrease of the microphonics.



F 49 *Diagrams of the destructions of the sensory cells, of the stria vascularis and of the gan-*
l cells in guinea pig N_2/1 L.



Fi . 50 Diagram of the destruction of the cochlear hai cells, plotted i black, in fi ars of guinea pigs treated with daily dose of 200 mg kg bod weight f kanamycin sulphat and of 15 mg kg bod weight of Nialamide for 30 da s.

A Cochlea

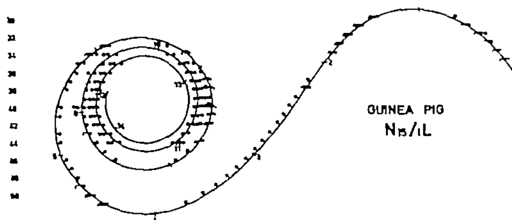
The sensory cells, especially the external hair cells, have disappeared for various distances from the beginning of the basal coil as represented in Fig. 50 The average distance over which all the external hair cells are missing is 5.02 mm in these animals examined, whereas the average distance to the point where all the external hair cells are present again is 8.02 mm (Table 22)

The severity of damage to the stria vascularis is proportionate to the extent of destruction of the external hair cells In the ear 10 L the stria vascularis has obviously been affected before the absence of the external hair cells however the latter do not look normal

Table 22 *Distances are given over which all the external hair cells have disappeared and the distances to the point where all the external hair cells are present again in the ears examined*

Th erage l es re calcul ted.

Guinea pigs Group N	Absence f th three E H C. (mm)	Presence of the three E H.C. (mm)
1 L	8.2	12.5
6 L	1	2.9
7 L	10.5	16.5
8 L	3.5	5.8
10 L	1 9	2.4
Average dist nce	.02	8.02



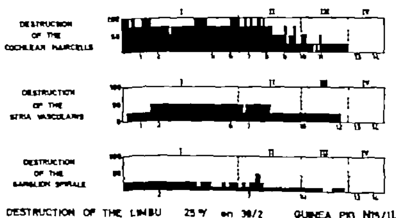
F o. 51. Diagram of the cochlear piral of the guinea pig N15/1 L with the remaining sensory cells. For the sak of clearness of the curve the cells still present ha been plotted only on the distance f impairment.

The ganglion spirale is completely normal in guinea pig 10 L. With guinea pig 6 L the impairment mainly consists of a lysis of certain ganglion cells in the ganglion spirale No. 1 With guinea pigs 8 L and 1 L it is clear that ganglion cells are missing in ganglion spirale No. 1 More towards the apex there is a lysis of the ganglion cells. With guinea pig 7 L up to 5 of the ganglion cells have disappeared, more in the ganglia of the apex than in the base or at least as many We shall return to this phenomenon later

The limbus is slightly affected with guinea pig 7 L, whereas with the other animals it is normal.

The surroundings are normal

The cochlear diagrams of the guinea pig N /1 L are fully represented in Figs. 51 and 52.



I 52 Diagrams of the destructions of the sensory cells, f the tria vascularis and of the ganglion cells with guinea pig N15/1 L.

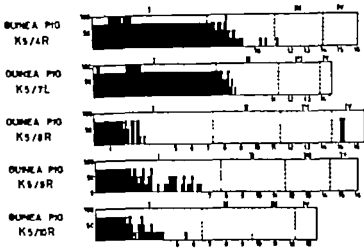


FIG. 53. Diagram of the destruction of the cochlear hai cells, plotted in black 1 fiv ears f guinea pigs tre ted with a daily dose f 250 mg kg body weight of ka amyci sulphate for 20 days.

B Vestibular system

The average duration of the postrotatory nystagmus in 17 animals stimulated by 10 revolutions in 20 sec clockwise and anticlockwise is 11.5 sec and 11 7 sec re pectively

From a histological point of view the vestibular system was fully normal with two animals. With the three others the following findings were established

The macula utriculi was slightly but distinctly impaired some sensory cells were expelled

Impairment of the ensory cells of the cristae though less strongly marked

The macula sacculi was normal.

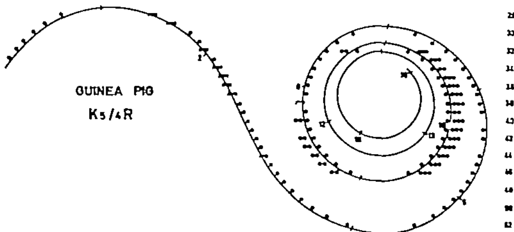
Group k_3

Guinea pigs treated with a daily dose of 250 mg/kg body weight of kanamycin sulphate for 20 days.

In this group the microphonics had dropped in 14 of the 15 ears. From this series of 14 ears we took five petrous bones at random for histological examination.

A *Cochlea*

The sensory cells, especially the external hair cells, have disappeared for variou distances fr m the beginning of the basal coil as represented in Fig 53 The average distance over which all the external hair cells are missing is 4 24 mm in these animals examined, whereas the average distance to the point where all the external hair cells are present again is



F a. 54 Diagram of the cochlear spiral of the guinea pig $K_5/4$ R with the remaining sensory cells. For the sak f clearness of the curv the cells still present have been plotted only on the di tance of impairment.

7 1 mm (Table 23) As in this group onl\ 14 animals showed a drop in their microphonics, a correcting factor has to be applied to this average distance (see Table 23) The corrected average distances are then 3 95 mm and 6 62 mm respectively

The severity of damage to the stria vascularis is proportionate to the extent of destruction of the external hair cells.

The ganglion spirale is hardly affected in guinea pigs 8 R, 9 R and 10 R. With guinea pig 7 L a disappearance of the ganglion cells is established to a degree of 25 % in ganglion No 1 With guinea pig 4 R we established that up to 50 % of the ganglion cells have disappeared in ganglion No 1 and up to 25 % in ganglion No 2

The limbus and the surroundings are unimpaired

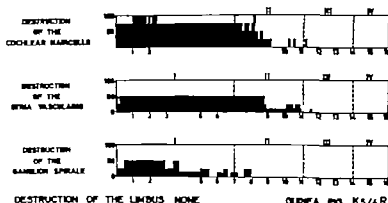


Fig. 55 Diagrams of the destructions of the sensory cells, of the stria vascularis d of the gangli lls w th guinea pig $K_5/4$ R.

TABLE 23 *Distances are given over which all the external hair cells have disappeared and the distances to the point where all the external hair cells are present again in the ears examined*

Th erag alues nd the corrected v lues are lculated

G inea pigs, Group K	Absence f the three E.H.C. (mm)	Presence of the three E.H.C (mm)
4 R	7.5	11.3
7 L	7 9	8.8
8 R	1.8	3.1
9 R	2.1	6.5
10 R	1 9	5.8
Average distance	4.21	7 1
After correction	3.05	6 62

Thi correction i calculated f llow : (mber f ormal im i impairment i 0 mm) + (number f i t i ted nimal erag di t nce) di ided by the total n mber f imal xamined lectrophy iologic lly So fter correction w fi d the foll wi g i f r the d st nce on which ll the ternal hai cell re missi g (4.21 mm):

(1 0) + (14 4.21) di ided by 15 = 3.95 mm.)

Th di tance t the poi t where ll the t rnal hai cell re present g i (7 1 mm) becomes fter correction:

(1 0) + (14 7 1) di ided b 15 = 6.62 mm.)

The cochlear diagrams of the guinea pig K /4 R are fully represented in Figs. 54 and 55

B Vestibular system

The average duration of the postrotatory nystagmus in 1, animals stimulated by 10 revolutions in 20 sec is 11.8 sec after clockwise as well as anticlockwise turning

From a histological point of view the vestibular system is completely normal in guinea pig 7 L and 8 R

In three other animals the following phenomena were established

Distinct impairment of the macula utriculi
Slight impairment of the cristae ampullares.
The macula acculi was affected only slightly if at all.

Croup P

Guinea pigs treated with a daily dose of 2o0 mg/kg body weight of kana mycin monopantothenate for 20 days.

In this group only 6 of the 15 ears showed a drop in the microphonics The five petrous bones that were examined histologically belonged to these six affected ears

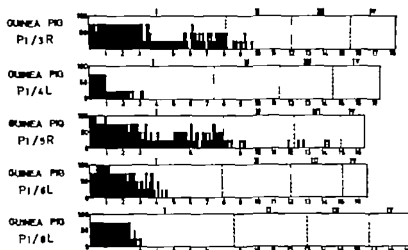


Fig. 56. Diagram of the destruction of the cochlear hair cells, plotted in black, in five ears of guinea pigs treated with daily dose of 250 mg/kg body weight of kanamycin monopantothenat for 20 days.

A *Cochlea*

The sensory cells, especially the external hair cells, have disappeared for various distances from the beginning of the basal coil as represented in Fig. 56 The average distance over which all the external hair cells are missing is 2.26 mm in these ears examined, whereas the average distance to the point where all the external hair cells are present again is 5.88 mm. In considering this, we have to take into account the fact that these five ears showed the most significant drop in the microphonics of the whole group in which only six ears were impaired whereas in all other groups the ears were chosen more at random. After correction the average distances become 0.9 mm and 2.35 mm respectively (Table 24)

Table 24 *Distances are given over which all the external hair cells have disappeared and the distances to the point where all the external hair cells are present again in the ears examined*

The average values and the corrected values are calculated.

Guinea pigs, Group P	Absence of the three E.H.C. (mm)	Presence of the three E.H.C. (mm)
3 R	3.2	9.5
4 L	1	3.1
5 R	2.4	9.3
6 L	2.3	4.5
8 L	2.4	3
Average distance	2.26	5.88
After correction	0.9	2.35

TABLE 23 *Distances are given over which all the external hair cells have disappeared and the distances to the point where all the external hair cells are present again in the ears examined*

The a erage l es nd the corrected alues are lculated.

Guinea pigs Croup K	Absence of the three E H C. (mm)	Presence f the three E H C. (mm)
4 R	7.5	11.3
L	.0	8.8
8 R	1 8	3.1
9 R	2.1	6..
10 R	1 9	5.8
Average dist nce	4.21	7 1
After correction	3 0.	6.02

Thi correction i calculated a f llow : (n mber f n rmal a im l impairment t 0 mm) + (umber of int lcated animal erag di t nce) divided by th tot l n mber f anim l xami ed lectrophysiologically So aft correction w find the f ll wing lue for th dist nce on which all th e ternal hair cell re mi i g (4.21 mm):

(1 0) + (14 4.21) divided by 15 = 3.93 mm.)

Th di t nce to the point where ll the ternal h ir cell are present gain (7 1 mm) becomes fter correcti n:

(1 0) + (14 7 1) di ided by 15 = 6.62 mm)

The cochlear diagrams of the guinea pig K /4 R are fully represented in Figs. 54 and 55

B Vestibular system

The average duration of the postrotatory nystagmus in 17 animals stimulated by 10 revolutions in 20 sec is 11.8 sec after clockwise as well as anticlockwise turning.

From a histological point of view the vestibular system is completely normal in guinea pig 7 L and 8 R

In three other animals the following phenomena were established

Distinct impairment of the macula utriculi
Slight impairment of the cristae ampullares.
The macula sacculi wa affected only slightly if at all.

Croup P_1

Guinea pigs treated with a daily dose of 250 mg/kg body weight of kanamycin monopantothenate for 20 days

In this group only 6 of the 15 ears showed a drop in the microphonics. The fi e petrous bones that were examined histologically belonged to these six affected ears.

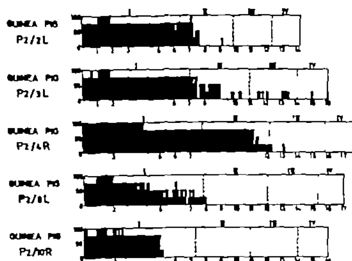


Fig. 50 Diagram of the destruction of the cochlear hair cells, plotted in black, in five ears of guinea pigs treated with daily dose of 250 mg/kg body weight of kanamycin dipantothenat for 20 days.

B. *Vestibular system*

The average duration of the postrotatory nystagmus in 17 animals stimulated by 10 revolutions in 20 sec clockwise and anticlockwise is 13.2 sec and 13.9 sec respectively

The histological examination revealed the following findings

The vestibular system is complet ly normal with guinea pig 8 L.

The macula utriculi is very slightly affected in guinea pig 4 L and 6 L, slightly more in guinea pig 3 R and se erely damaged in gui ea pig 5 R.

The cristae ampullares re norm l in guinea pig 4 L and 6 L, slightly damaged in guinea pig 3 R and 5 R.

The macula sacculi is normal with all the ears examined

Group P_2

Guinea pigs treated with a daily dose of 250 mg/kg body weight of kanamycin dipantothenate for 20 days.

In this group 10 out of the 15 ears showed a drop in the microphonics. The fi e petrous bones that were examined histologically belonged to these ten affected animals.

A *Cochlea*

The sensory cells, especially the external hair cells, have disappeared for arious distances from the beginning of the basal coil as represented in Fig 59 The average distance over wich all the external hair cells are mis ing i 6 3 mm for these ears examined, whereas the average distance to the point where all the external hair cells are present again is 9 48 mm.

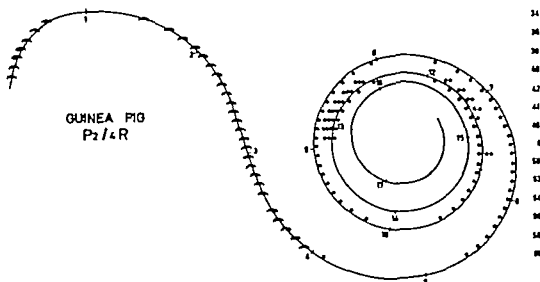


FIG. 60. Diagra of the cochlear spiral of the guinea pig P 4 R with the remaini g sensory cells. For the sake of clearness of the curve the cells till present have been plotted only on the dist nce of impairment

After correction these average distances become 4 2 mm and 6 32 mm respectively (Table 25)

The stria vascularis is markedly affected with all these animals in proportion to the extent of destruction of the external hair cells, as described for group K_e.

The ganglion spirale is normal in guinea pig 2 L, whereas it is more affected in the other animals, especially in the basal ganglia Guinea pig 4 R shows a loss of ganglion cells up to 75% in ganglion spirale No 1 (Fig 61)

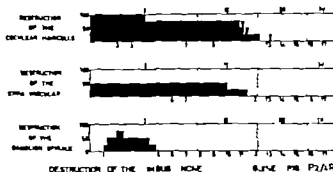
The limbus and the surroundings are normal

The cochlear diagrams of the guinea pig P_2/4 R are fully represented in Figs 60 and 61

TABLE 25 *Distances are given over which all the external hair cells have disappeared and the distances to the point where all the external hair cells are present again in the ears examined*

The erage v lues nd th corrected l e re calculated.

G inea pigs, Group P	Absence f the three I H.C. (mm)	Presence f the three I H.C. (mm)
2 L	6	7 8
3 L	0.1	13.5
4 R	10 9	13.1
8 L	3.5	8
10 R	5	5.2
A erag dist nce	6 3	9 48
After correction	4 2	6.32



Fi 61 Diagrams of the destructions of the sensory cells, of the stria vascularis and of the ganglion cells with guinea pig P_2/4 R.

B Vestibular system

The average duration of the postrotatory nystagmus in 16 animals stimulated by 10 revolutions in 20 sec clockwise and anticlockwise is 13 sec and 13.5 sec respectively

Histologically the vestibular system is completely normal in guinea pig 2 L and 3 L. In the other animals the following findings are established

The macula utriculi shows early ffection in guinea pig 8 L a d 4 R a severe damage in guinea pig 10 R

The cristae mpullares are slightly affected in guinea pig 8 L, 4 R and 10 R.
The macula sacculi is normal with ll the ears xamined

Group P

Guinea pigs treated with a daily dose of 250 mg/kg body weight of kanamycin tripantothenate for 20 days.

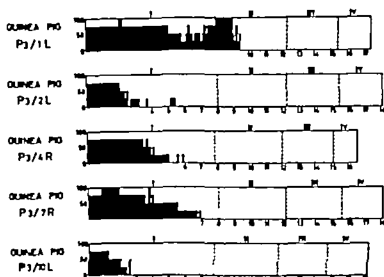
In this group 11 out of the 15 ears showed a drop in the microphonics. The five petrous bones that were examined histologically belonged to these eleven ears.

A Cochlea

The sensory cells, especially the external hair cells, have disappeared for various distances from the beginning of the basal coil as represented in Fig 62 The average distance over which all the external hair cells are missing is 3 mm in these five animals examined whereas the average distance to the point on which all the external hair cells are present again s 6 mm. After correction these average distances become 2.2 mm and 4 4 mm respectively (Table 20)

The severity of damage to the stria vascularis is proportionate to the xtent of destruction of the external hair cells.

Th ganglion spirale is slightly affected in three animals. In guinea pig



Fi 62. Diagram of the destruction of the cochlea hair cells, plotted in black, f fi ears of guinea pigs treated with daily dose f 250 mg kg body weight of kanamycin tripa tothenat for 20 days.

4 R there is a loss of ganglion cells up to 25% in ganglion spirale No 1 In guinea pig 1 L there is a loss of 50 of ganglion cells in ganglion spirale No 3, corresponding to the destruction of the organ of Corti No 3 (Fig 62)

The limbus is distinctly affected in guinea pig 1 L, while in the other animals it is normal

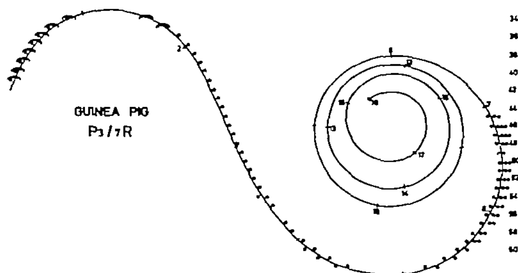
The surroundings are normal

The cochlear diagrams of the guinea pig P /7 R are fully represented in Figs. 63 and 64

TABLE 26 *Distances are given over which all the external hair cells have disappeared and the distances to the point where all the external hair cells are present again in the ears examined*

The rage lues and the corrected lues re calcul ted.

C inea pigs, Group P	Absence f the three E H C. (mm)	Presence of the three E.H.C. (mm)
1 I	6	9.2
2 L	2	5.6
4 R	3.3	5.8
7 R	3.5	6.9
10 I	1 2	2.5
Averag distance	3	6
After correction	2.2	4.4



F o. 63. Diagram of the cochlear spiral f th guinea pig $P_3/7$ R with the remaining sensory cells. For the sak of clearness of the curve the cells till present have been plotted only on the distance of impairment.

B Vestibular system

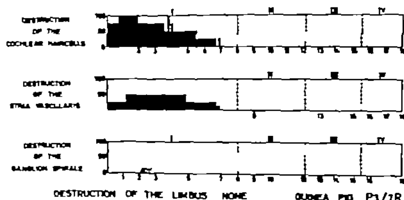
The average duration of the postrotatory nystagmus in 17 animals stimulated by 10 revolutions in 20 sec, clockwise and anticlockwise is 13 9 sec and 13.5 sec respectively

Histologically the vestibular system is completely normal in guinea pig 1 L. With the other animals the following findings are established

The macula utriculi is norm l in guinea pig 2 L, lightly affected in guinea pig 7 R and 10 L, distinctly impaired in guinea pig 4 R.

The c ista ampullares are normal in guinea pig 7 R and 10 L, lightly affected in guinea pig 2 L and distinctly impaired in guinea pig 4 R.

Th macula sacc li is normal in all the ears examined.



F 64 D grams of the destructions of the sensory cells, of the tria ascularis and of the ganglion lls with guinea pig $P_3/7$ R.

3 STREPTOMYCIN—VIOMYCIN—CAPREOMYCIN

Two groups of guinea pigs were injected subcutaneously five days a week with streptomycin sulphate Group S_1 was given a daily dose of 100 mg/kg body weight for 75 days, total dose 7.5 g/kg body weight Group S_2 was given a daily dose of 200 mg/kg body weight for 58 days. Then the daily dose was doubled because no hearing defect appeared (Preyer reflex) Four animals died from acute streptomycin intoxication in two days, and the general condition of the rest of the animals became deteriorated After four days the double dose was stopped and the injections continued according to the original schedule up to a total dose of 15 g/kg body weight

Two other groups of guinea pigs were injected subcutaneously five days a week with viomycin sulphate Group V_1 was given a daily dose of 100 mg kg body weight for 75 days, total dose 7.5 g/kg body weight. Group V_2 was injected with a daily dose of 200 mg/kg body weight for 58 days and because impairment of hearing did not appear we changed over to a daily dose of 400 mg/kg body weight for 9 days, up to a total dose of 15 g/kg body weight

Finally the last two groups of guinea pigs were injected subcutaneously five days a week with capreomycin sulphate Group C_1 was given a daily dose of 100 mg kg body weight for 75 days making a total dose of 7.5 g/kg body weight Group C_2 was injected with a daily dose of 200 mg/kg body weight for 58 days and later 400 mg kg body weight for 9 days, because no hearing disorder had appeared after the 58 days, making a total dose of 15 g kg body weight

A control group was given a daily dose of 0.25 cc isotonic saline subcutaneously

The general condition remained good in all groups, except in group S_2, where five animals (25) died before the end of the injection period (Table 27) Three weeks after the last injection the microphonics of 15 ears in each group were recorded

I Cochlear microphonics

A total dose of 7.5 g/kg body weight of the three antibiotics in these groups has hardly any influence on the microphonics with the guinea pigs (Fig 65)

With the double total dose of 15 g kg body weight there is however a distinct drop in the viomycin group (Fig 66) In the streptomycin group there is a statistically significant drop at only one of the 18 points examined

(Table 35) and nowhere at all in the capreomycin group. Of these three antibiotics capreomycin seems to be the least toxic, whereas viomycin seems to be the most toxic for the microphonics with the guinea pigs (Fig. 66).

II. Histological results

Group S_1

Guinea pigs treated with a daily dose of 100 mg/kg body weight of streptomycin sulphate for 75 days.

This group was not examined histologically since no anomalies in the microphonics were established.

Group S_2

Guinea pigs treated with a daily dose of 200 mg/kg body weight of streptomycin sulphate to a total dose of 15 g/kg body weight.

In this group 11 out of the 15 ears showed a drop in the microphonics at the 10 000 c/s recording. The five ears that were examined histologically belonged to these eleven affected ears. Many animals showed symptoms of inflammation of the middle ear.

A. *Cochlea*

In three ears all the external hair cells were present, though near the beginning of the basal coil a few cells seemed to have been damaged. In the fourth coil the most medial external hair cell often showed a bulb shaped swelling with a condensation body in the supranuclear part (cell type A, Fig. 12).

In one ear all the external hair cells had disappeared over 0.24 mm, and they were fully present again at 0.32 mm. In guinea pig 8 R there was impairment up to 0.08 mm (Table 38).

The calculation of an average area of impairment with a correction according to the number of affected animals is not justified with this group,

Table 27. *Mortality rate and weight evolution during the test*

Animal group	No. of animals alive: On first injection	No. of animals alive: At moment of recording	Average weight: On first injection, g	Average weight: At moment of recording, g
S_1 (streptomycin sulphate 7.5 g/kg)	15	15	251.8	647.0
S_2 streptomycin sulphate 15 g/kg)	20	15	218.7	707.1
V_1 (viomycin sulphate 7.5 g/kg)	15	15	233.7	668.2
V_2 viomycin sulphate 15 g/kg)	20	19	231.0	711.0
C_1 (capreomycin sulphate 7.5 g/kg)	15	15	212.6	633.2
C_2 capreomycin sulphate 15 g/kg)	20	19	272.2	708.6
Isot. saline	15	15	216.5	698.3

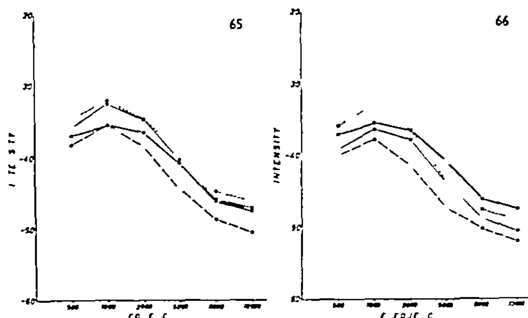


Fig. 65 Comparison between the 15 ears of the isotonic saline group and those of the S_1 (streptomycin sulphat 7.5 g kg), V (viomycin sulphate 7.5 g kg) and C_1 (capreomycin sulphat 7.5 g kg) groups at 100 db stimulation intensity —— isotonic saline group —— S group; — — — V group — — C_1 group.

Fig. 66. Comparison between the 15 ears of the isotonic saline group and those of the S_2 (streptomycin sulphat 15 g/kg), V (viomycin sulphate 15 g/kg) and C_2 (capreomycin sulphat 15 g kg) groups at 100 db stimulation intensity —— isotonic saline group —— S_2 group; — — — V group — C_2 group

since a lot of animals showed symptoms of inflammation of the middle ear

The stria vascularis was only slightly affected at the basal coil of guinea pig 1 R and 8 R

The number of ganglion cells seemed slightly diminished near the ganglion spirale No 1 in guinea pig 4 R, 6 R and 8 R

The limbus and the surroundings were normal

The cochlear diagrams of the guinea pig S_2/8 R are fully represented in Fig. 67

B *Vestibular system*

Functional examination the vestibular system was examined every week in the S_2 group The following data were noted symptoms of ataxia (walking at normal attitudes purposeless spinning round) the labyrinthine righting reflex, the perrotatory and postrotatory deviations and the duration of the postrotatory nystagmus after clockwise and anticlockwise rotation

The first symptoms of ataxia appeared with a total dose of 4 g/kg body weight of streptomycin and increased with 5 g kg body weight. After a dose of 6 g kg body weight three animals showed complete inexcitability

TABLE 28 *Average reaction of the 15 ears of the isotonic saline group*

The value of [illegible] is given in parentheses. This group was used as control.

Frequency c/s	80 db	90 db	100 db
500	44.06 (±9.81)	40.40 (±9.32)	36.86 (± .61)
1,000	42.86 (±8.94)	38.14 (±8.24)	33.26 (± .34)
2,000	35.73 (±6.85)	36.53 (±6.16)	36.33 (±5.74)
5,000	45.00 (±5.83)	42.80 (±5.91)	40.73 (±5.19)
8,000	52.33 (±4.58)	49.00 (±5.19)	46.00 (±5.4[illegible])
10,000	52.20 (±4.33)	50.06 (±4.[illegible]9)	4[illegible].40 (±5.0[illegible])

TABLE 29 *Average reaction of the 15 ears of the S group*

The value of [illegible] is given in parentheses.

Frequency c/s	80 db	90 db	100 db
[illegible]00	44.40 (±8.88)	40.20 (±9.64)	35.73 (± [illegible]00)
1,000	43.20 (±8.36)	35.40 (±8.91)	32.26 (±6.00)
2,000	37.26 (±6.78)	35.06 (±5.56)	34.46 (±5.00)
5,000	43.60 (±6.24)	40.11 (±5.43)	40.20 (±6.00)
8,000	51.93 (±5.00)	48.46 (±6.08)	43.93 (±6.48)
10,000	52.60 (±4.58)	49.46 (±5.91)	47.00 (±5.65)

TABLE 30 *Average reaction of the 15 ears of the S_2 group*

The value of [illegible] is given in parentheses.

Frequency	80 db	90 db	100 db
500	47.06 (±8.88)	43.20 (±9.21)	39.13 (±8.54)
1,000	48.06 (±8 [illegible]1)	41.53 (±9.64)	36.06 (± [illegible].68)
2,000	41.26 (±7.21)	35.60 (±6.48)	37.73 (±5.00)
5,000	48.46 (±5.65)	45.20 (±6.16)	43.73 (±5.[illegible]4)
8,000	55.13 (±2.49)	51.80 (±4.79)	48.60 (±5.00)
10,000	56.13 (±3.00)	53.26 (±4.00)	50.46 (±4.47)

TABLE 31 *Average reaction of the 15 ears of the V_1 group*

The value of [illegible] is given in parentheses.

Frequency c/s	80 db	90 db	100 db
500	43.86 (±8.6[illegible])	40.66 (±8.71)	39.13 (±7.81)
1,000	44.13 (±8.12)	39.46 (±8.54)	35.20 (±6.65)
2,0[illegible]	41.86 (±6.70)	39.20 (±5.91)	38.40 (±4.35)
5.[illegible]	49.33 (±5.00)	45.73 (±4.89)	44.26 (±5.00)
8[illegible]	55.00 (±3.31)	51.13 (±3.87)	49.60 (±4.35)
[illegible]	51.86 (±3.31)	52.06 (±4.00)	50.40 (±3.[illegible]4)

Table 32. *Average reaction of the 15 ears of the S_2 group*

The value f l gi en in parentheses.

Frequency c/s	80 db	90 db	100 db
500	11.13 (±7.93)	42.00 (±8.60)	39.93 (±6.83)
1,000	45.13 (±8.00)	40.60 (± .81)	37 06 (±6.70)
2,000	42.60 (±6.00)	40.73 (±6.00)	41.20 (±6.70)
5,000	49.33 (±4.80)	47 46 (±5.83)	47 13 (±6.16)
8,000	51 73 (±3.46)	52.00 (±4.38)	50.13 (±5.19)
10 000	55 13 (±3.00)	52.73 (±1.21)	51.93 (±5.19)

Table 33 *Average reaction of the 15 ears of the C_1 group*

Th alue f is gi en i pare theses.

Frequency c/s	80 db	90 db	100 db
500	41 73 (±10 00)	38.01 (±10.18)	31 40 (±8.00)
1 000	42.00 (± 8.36)	36.40 (± 8.51)	31.86 (±6.55)
2,000	39.26 (± 8 66)	36.26 (± 8 12)	31 00 (±6.63)
5,000	41.26 (± 14)	41.53 (± 6.63)	39.26 (±5.19)
8,000	52.26 (± 5.09)	48.80 (± 5.91)	41 60 (±5.20)
10,000	52.60 (± 1.21)	49.33 (± 5.33)	46.00 (±4.79)

Table 34 *Average reaction of the 15 ears of the C_2 group*

Th v l of f given in parentheses.

Frequency c/s	80 db	90 db	100 db
500	41 12 (±7.81)	37 93 (±7 41)	33.66 (±6.53)
1,000	41.00 (±6.70)	35.73 (±7.07)	32.60 (±6.40)
2,000	38.93 (±6.83)	36.73 (± 14)	36.33 (±6.85)
5,000	47 13 (±5.29)	41 73 (±6.40)	43.46 (±6.16)
8,000	53.00 (±3.60)	48 60 (±4.69)	47 40 (±5.65)
10,000	53.73 (±3.60)	50.80 (±4.58)	48.86 (±5.19)

Table 35 *Value of F (1.28) where the reaction is statistically better in the normal animals (isotonic saline group) than in the S_2 group on the 18 examined points*

Test 15 ears, Italic type $P < 0.01$

Frequency c/s	80 db	90 db	100 db
500	—	—	—
1,000	—	—	—
2,000	—	—	—
5,000	—	—	—
8,000	—	—	—
10,000	f	—	—

TABLE 36 *Value of F (1.28) where the reaction is statistically better in the normal animals (isotonic saline group) than in the V_2 group on the 18 examined points*

Test on 18 ears Italic type $P < 0.01$ Roman type $0.01 < P < 0.05$

Frequency c/s	80 db	90 db	100 db
500	—	—	—
1,000	—	—	—
2,000	—	—	4.54
5,000	4.93	4.75	[illegible]
8,000	—	—	4.53
10,000	4.60	—	5.91

TABLE 37 *Value of F (1.28) where the reaction is statistically better in the C_2 than in the V_2 group on the 18 examined points*

Test on 18 ears. Roman type $0.01 < P < 0.05$

Frequency c/s	80 db	90 db	100 db
500	—	—	—
1,000	—	—	4.45
2,000	—	—	—
5,000	—	—	—
8,000	—	—	—
10,000	—	—	—

TABLE 38 *Distances are given over which all the external hair cells have disappeared and the distances to the point where all the external hair cells are present again in the ears examined*

Guinea pigs, Group S_1	Absence of the three E.H.C. (mm)	Presence of the three E.H.C. (mm)
1 R	0	0
4 R	0	0
6 R	0.24	0.32
8 R	0	0.08
9 L	0	0

f the vestibular system during the rotating test this was accompanied by the absence of the perrotatory and the postrotatory deviation and of the labyrinthine righting reflex and by strong ataxia symptoms.

When more of the antibiotic was administered the ataxia symptoms increased in several animals four animals were inexcitable by the rotating

TABLE 32 *Average reaction of the 15 ears of the V_2 group*

The value of s is given in parentheses.

Frequency c/s	80 db	90 db	100 db
500	41 13 (± 93)	42.00 (±8.00)	39.93 (±6.85)
1,000	45.13 (±8.00)	40 60 (±7.81)	37.06 (±6.70)
2,000	42 00 (±6.00)	40.73 (±6.00)	41.20 (±6.70)
5,000	49.33 (±4 80)	47 40 (±5.83)	47 13 (±6.16)
8,000	51 73 (±3.46)	52.00 (±1.58)	50.13 (±5.10)
10,000	55 13 (±3.00)	52.73 (±1.21)	51.03 (±5.19)

TABLE 33 *Average reaction of the 15 ears of the C_1 group*

The value of s is given in parentheses.

Frequency	80 db	90 db	100 db
500	41 73 (±10 00)	38.01 (±10 48)	31 40 (±8.00)
1 000	42.00 (± 8.36)	36.40 (± 8.51)	31.80 (±6.55)
2,000	39.20 (± 8 06)	36.26 (± 8 12)	31 60 (±6.63)
5,000	41.26 (± 7 14)	41.53 (± 6.03)	30.26 (±5.19)
8 000	52.26 (± 5.00)	48.80 (± 5 91)	41.60 (±5.20)
10 000	52.60 (± 1.21)	50.33 (± 5.38)	46.00 (±4 79)

TABLE 34 *Average reaction of the 15 ears of the C_2 group*

The value of s is given in parentheses.

Frequency c	80 db	90 db	100 db
500	41 12 (±7.81)	37.93 (±7 11)	35.66 (±6.55)
1 000	41 06 (±6.70)	33 73 (±7 07)	32.60 (±6.10)
2,000	38.03 (±6.33)	30.73 (±7 14)	36.33 (±6.85)
5 000	47 13 (±5.29)	41 73 (±6.10)	43.46 (±6.16)
8 000	53.00 (±3.60)	49 60 (±1.89)	47 40 (±5 63)
10,000	53.73 (±3.60)	50.80 (±1.58)	48.66 (±5.19)

TABLE 35 *Value of F (1.28) where the reaction is statistically better in the normal animals (isotonic saline group) than in the S_2 group on the 18 examined points*

Test on 15 ears. It lle type P 0.01

Frequency c/s	80 db	90 db	100 db
500	—	—	—
1,000	—	—	—
2,000	—	—	—
5,000	—	—	—
8,000	—	—	—
10,000	8 22	—	—

Group V₁

Guinea pigs treated with a daily dose of 100 mg/kg body weight of viomycin sulphate for 75 days.

This group was not examined histologically since no anomalies in the microphonics were established

Group V₂

Guinea pigs treated with a daily dose of 200 mg/kg body weight of viomycin sulphate for 58 days followed by a daily dose of 400 mg/kg body weight of viomycin sulphate for 9 days to a total dose of 15 g/kg body weight

In this group we established a drop in the microphonics in 9 out of the 15 ears The five ears that were examined histologically belonged to these nine affected animals.

A *Cochlea*

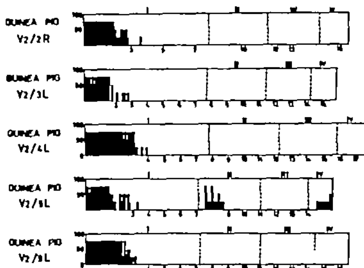
In all the animals the external hair cells in the basal coil were affected for various distances. The diagram of the missing sensory cells is represented in Fig 68 The average distance over which all the external hair cells are missing is 1 94 mm in the animals examined whereas the average distance to the point on which all the external hair cells reappear is 3.3 mm. The unusual picture of intoxication in guinea pig 5 L is not taken into account. After correction these average distances become 1 1 mm and 1 98 mm respectively (Table 40)

The stria vascularis is also affected in the areas, corresponding to those with injured external hair cells.

TABLE 40 *Distances are given over which all the external hair cells have disappeared and the distances to the point where all the external hair cells reappear in the ears examined*

The average values and the corrected values are calculated.

Guinea pigs, Group V	Absence of the three E.H.C. (mm)	Presence of the three E.H.C. (mm)
2 R	1.9	3 7
3 L	1.6	2.6
4 L	2.4	3.8
5 L	1.5	3.3
9 L	2.3	3.1
Average distance	1.94	3.3
After correction	1 1	1.98



F G. 68. Diagram of the destruction of the cochlear hair cells, plotted in black, i fiv ears of guinea pigs treated with viomycin t a t tal dose of 15 g/kg body weight.

The ganglion cells are slightly affected to a maximum loss of 25% in one animal in ganglion spirale No 1 In the other ganglia the cells are normal except in guinea pig 5 L. This strange intoxication picture will be discussed later

The limbus and the surroundings are normal

The cochlear diagrams of guinea pig $V_2/4$ L are fully represented in Figs. 69 and 70

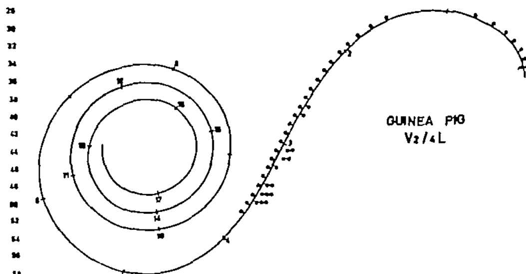


FIG. 69 Diagram of the cochlear spiral of the guinea pig $V_2/4$ L with the remaining sensory cells. For the sak f learness f the curve th cell till present h ve been plotted only on th distance of impairment.

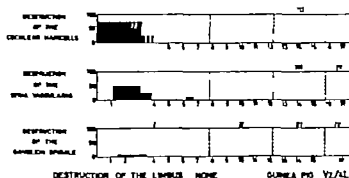


Fig. 70 Diagrams of the destructions of the sensory cells, of the stria vascularis and of the ganglion cells with guinea pig VI/4 L.

B Vestibular system

The average duration of the postrotatory nystagmus in 17 animals stimulated by 10 revolutions in 20 sec clockwise and anticlockwise is 12.4 sec and 12.7 sec respectively

Histologically the vestibular systems in guinea pig 3 L and 4 L are completely normal In the other animals the findings are as follows

The macula utriculi is slightly impaired

The cristae ampullares are normal in guinea pig R and slightly affected in guinea pig 5 L and 9 L.

The macula sacculi is normal in all the ears examined

Group C_1

Guinea pigs treated with a daily dose of 100 mg/kg body weight of capreomycin sulphate for 15 days.

This group was not examined histologically since no anomalies in the microphonics were established

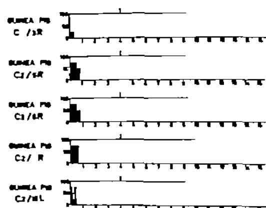


Fig. 71 Diagram of the destruction of the cochlear hair cells, plotted in black in f guinea pigs treated with capreomycin to total dose of 15 g/kg body weight.

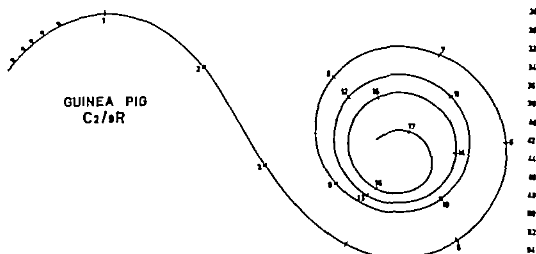


Fig. 72. Diagram of the cochlear spiral of the guinea pig C_3/9 R with the remaining sensory cells. For the sake of clearness of the curve the cells still present have been plotted only on the distance of impairment

Group C_3

Guinea pigs treated with a daily dose of 200 mg/kg body weight of capreomycin sulphate for 58 days, followed by 400 mg/kg body weight for 9 days to a total dose of 15 g/kg body weight

In this group a drop in the microphonics was seen in 7 out of the 15 ears. The five ears that were examined histologically were among these seven affected animals

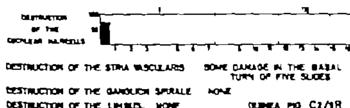
A. Cochlea

The external hair cells in the basal coil were very slightly impaired The diagram of the missing sensory cells of the five ears is represented in Fig 71 The average distance over which all the external hair cells are missing

TABLE 41 *Distances are given over which all the external hair cells have disappeared and the distances to the point where all the external hair cells reappear in the ears examined*

The average values and the corrected values are calculated.

Guinea pigs, Group C_4	Absence of the three E.H.C. (mm)	Presence of the three E.H.C. (mm)
3 R	0	0.22
5 R	0.5	0.8
6 R	0.5	0.8
9 R	0.6	0.6
10 L	0.2	0.5
Average distance	0.36	0.6
After correction	0.16	0.28



F g. 73 Diagram f the destruction f the sensory cells with guinea pig C_7 9 R.

is, in these ears examined, 0.36 mm, whereas the average distance to the point where all the external hair cells reappear is 0.6 mm. After correction these average distances become respectively 0 16 mm and 0.28 mm (Table 41)

The stria vascularis is very slightly affected.

The ganglion cells, the limbus and the surroundings are completely normal.

The cochlear diagrams of guinea pig C_7 9 R are fully represented in Figs. 72 and 73.

B Vestibular system

The average duration of the postrotatory nystagmus in 1 animals stimu lated by 10 revolutions in 20 sec clockwise and anticlockwise is 11.8 sec and 11.9 sec respectively

All the vestibular system are histologically entirely normal

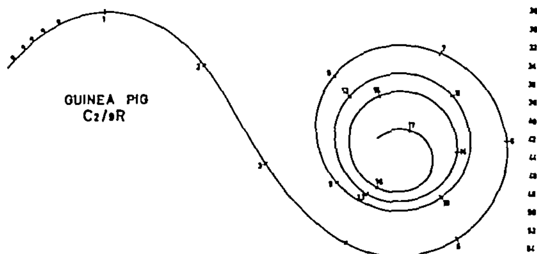


FIG. 72. Diagram of the cochlear spiral of the guinea pig $C_2/9$ R with the remaining sensory cells. For the sake of clearness of the curve the cells still present have been plotted only on the distance of impairment.

Group C_2

Guinea pigs treated with a daily dose of 200 mg/kg body weight of capreomycin sulphate for 58 days, followed by 400 mg/kg body weight for 9 days to a total dose of 15 g/kg body weight

In this group a drop in the microphonics was seen in 7 out of the 15 ears. The five ears that were examined histologically were among these seven affected animals.

A. *Cochlea*

The external hair cells in the basal coil were very slightly impaired The diagram of the missing sensory cells of the five ears is represented in Fig 71 The average distance over which all the external hair cells are missing

TABLE 41 *Distances are given over which all the external hair cells have disappeared and the distances to the point where all the external hair cells reappear in the ears examined*

The average values and the corrected values are calculated.

Guinea pigs, Group C_2	Absence of the three E.H.C. (mm)	Presence of the three E.H.C. (mm)
3 R	0	0.32
5 R	0.5	0.8
8 R	0.5	0.8
9 R	0.6	0.6
10 L	0.2	0.5
Average distance	0.36	0.6
After correction	0.16	0.28

TABLE 42. *Mortality rate and weight evolution during the test*

Animal group	No of animals alive		Average weight	
	On first injection	At moment of recording	On first injection, g	At moment of recording, g
Neo_3 (neomycin sulphate 3 g/kg)	15	15	353.2	782.6
Methylneomycin (3 g/kg)	15	15	335.6	732.6
Neo_1 (neomycin sulphate 1.5 g/kg)	10	10	215.3	414.5
Neo_2 (neomycin sulphate 2 g/kg)	10	10	253.5	331.5
Neo_2 B (neomycin sulphate 2 g/kg + Vit. B)	10	10	216.0	317.5
Isotonic saline	16	15	362.4	794.6

TABLE 43. *Average reaction of the 15 ears of the isotonic saline group*

The value of σ is given in parentheses. This group was used as control.

Frequency c/s	80 db	90 db	100 db
500	44.60 (±10.72)	39.53 (±11.09)	35.80 (±9.05)
1,000	41.40 (±10.34)	36.26 (±9.74)	32.53 (±6.70)
2,000	38.60 (±8.06)	31.86 (±6.40)	33.93 (±4.58)
5,000	42.66 (±7.87)	39.40 (±5.83)	38.86 (±4.80)
8,000	46.53 (±6.63)	43.46 (±6.08)	43.13 (±1.47)
10,000	49.06 (±6.00)	46.60 (±5.56)	45.93 (±4.24)

TABLE 44. *Average reaction of the 15 ears of the* Neo_3 *group*

The value of σ is given in parentheses.

Frequency c/s	80 db	90 db	100 db
500	59.13 (±2.44)	58.93 (±2.82)	58.66 (±3.46)
1,000	58.93 (±2.82)	58.80 (±3.16)	58.93 (±2.82)
2,000	59.26 (±2.00)	59.06 (±2.44)	58.86 (±3.00)
5,000	59.73 (±1.00)	59.73 (±1.00)	59.66 (±1.41)
8,000	59.80 (±1.41)	60.00 (±0.00)	60.00 (±0.00)
10,000	60.00 (±0.00)	60.00 (±0.00)	60.00 (±0.00)

TABLE 45. *Average reaction of the 15 ears of the methylneomycin group (3 g/kg)*

The value of σ is given in parentheses.

Frequency c/s	80 db	90 db	100 db
500	44.11 (±9.64)	39.46 (±9.79)	36.46 (±8.60)
1,000	41.40 (±8.77)	36.33 (±8.42)	33.46 (±7.21)
2,000	41.6[illegible] (±7.54)	37.00 (±6.55)	36.20 (±5.65)
5,000	43.06 (±6.55)	41.00 (±5.65)	41.40 (±4.69)
8,000	45.26 (±6.09)	45.93 (±5.56)	45.13 (±4.12)
10,000	49.93 (±3.47)	49.33 (±5.09)	48.00 (±4.33)

TABLE 46 *Average reaction of the 10 ears of the $Neo_{1,3}$ group*

The v lue f 1 given in parentheses.

Frequency c/s	80 db	90 db	100 db
500	41.30 (±5.74)	41.50 (±6.48)	40.30 (±6.55)
1,000	41 40 (±6.00)	41 00 (±7.81)	38.80 (±8.12)
2,000	42.00 (±6.70)	42.00 (±6.85)	42.60 (±6.18)
5,000	50.20 (±5.71)	48.90 (±5.74)	48.30 (±6.08)
8,000	51 10 (±3.16)	52.40 (±4 17)	51 40 (±5.00)
10,000	51 90 (±3.16)	53.40 (±4.00)	52.70 (±4.58)

TABLE 47 *Average reaction of the 10 ears of the Neo_2 group*

The v lu f 1 gi n i parentheses.

Frequency c/s	80 db	90 db	100 db
500	50.20 (±5.38)	47 70 (±5.63)	45.10 (±5.29)
1,000	49 60 (±6.21)	46.50 (±7.00)	45.00 (±6.21)
2,000	49.20 (±6.48)	47 10 (±5. 4)	47.80 (±5.09)
5,000	55.30 (±4 17)	51 10 (±4.89)	53.10 (±4.89)
8,000	57 10 (±3.46)	56.60 (±4.00)	51 80 (±3.74)
10,000	57.90 (±2.01)	56.70 (±3.60)	56.00 (±2.81)

TABLE 48 *Average reaction of the 10 ears of the $Neo_{2,B}$ group*

The 1 f 1 given i parentheses.

Frequency c/s	80 db	90 db	100 db
500	52.40 (±3.71)	50.80 (±4 47)	48.70 (±5.00)
1,000	53.20 (±3.71)	50.70 (±4.89)	49 10 (±5.38)
2,000	52 10 (±4 12)	50.60 (±4.58)	50.20 (±4 79)
5,000	57.30 (±2.00)	56.00 (±2.61)	51.90 (±3.31)
8,000	58.30 (±1 73)	57.50 (±2.00)	56.90 (±1 73)
10,000	58.70 (±1 41)	58.10 (±1 73)	57 00 (±2.23)

TABLE 49 *Value of F (1.23) where the reaction is statistically better in the control animals (isotonic saline group) than in the $Neo_{1,3}$ group on the 18 examined points*

Test 10 rs. It 11 type $P < 0.01$ R man type $0.01 < P < 0.05$

Frequency c/s	80 db	90 db	100 db
500	—	—	—
1,000	—	—	4 12
2,000	—	7.02	*16.49*
5,000	6.35	*16.18*	*14 37*
8,000	*12.00*	*14 42*	*18 38*
10,000	*7 82*	*10 89*	*11 06*

TABLE 50 *Value of F (1.23) where the reaction is statistically better in the control animals (isotonic saline group) than in the Neo_3 group on the 18 examined points*

Test on 10 ears. Italic type $P < 0.01$ Roman type $0.01 < P < 0.05$.

Frequency c/s	80 db	90 db	100 db
500	—	4.56	*8.04*
1,000	5.02	*8.47*	*31.76*
2,000	*11.61*	*31.70*	*50.36*
5,000	*20.32*	*42.92*	*73.30*
8,000	*22.33*	*31.02*	*46.11*
10,000	*19.01*	*25.33*	*43.83*

TABLE 51 *Value of F (1.23) where the reaction is statistically better in the control animals (isotonic saline group) than in the $Neo_{2\,B}$ group on the 18 examined points*

Test on 10 ears. Italic type $P < 0.01$ Roman type $0.01 < P < 0.05$

Frequency c/s	80 db	90 db	100 db
500	4.86	*9.21*	*16.68*
1,000	*11.79*	*18.60*	*42.61*
2,000	*23.97*	*44.64*	*72.19*
5,000	*31.31*	*70.86*	*82.96*
8,000	*20.32*	*40.54*	*83.01*
10,000	*21.49*	*38.88*	*50.53*

II HISTOLOGICAL RESULTS

Among the animals treated with neomycin and methylneomycin only the group Neo and Methylneo were examined histologically.

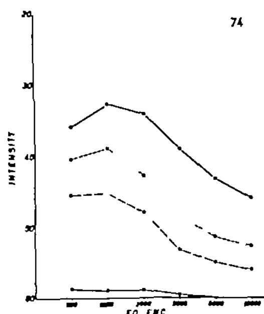
Group Neo_3

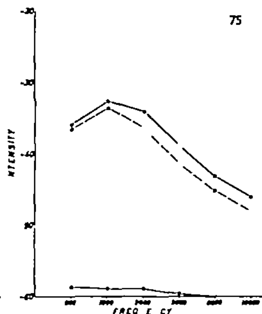
Guinea pigs treated with a daily dose of 100 mg/kg body weight of neomycin sulphate for 30 days.

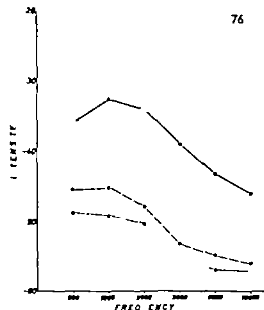
All animals showed nearly 100% drop in the microphonics. Ten cochleae from six animals and six vestibular systems from four animals were fully examined histologically.

A *Cochlea*

The organ of Corti had generally disappeared over the total length of the basal coil and had been replaced by undifferentiated epithelium, which had equally replaced the cells of Claudius. The internal hair cells appeared again about the first half of the second coil and the external hair cells



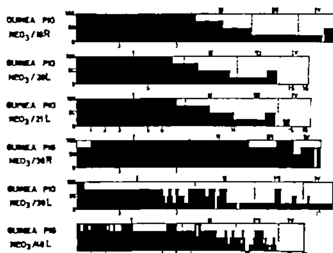




F 74 Comparison between the 15 ears of the isotonic salin group and those of the Neo₃ (neom cin ulphat 3 g/kg), Neo₂ (neomycin sulphat 2 g/kg—10 ears) and Neo₁.₅ (neomycin sul ph t 1 5 g/kg—10 ears) groups at 100 db stimulation intensity —— isotonic saline group; —— Neo₃ group — — Neo₂ group. Neo₁ group.

Fig 75 Comparison between the 15 ears f th isotonic saline group and those of the Neo₃ (neomycin sulphat 3 g/kg) and methylneomyci (3 g/kg) groups t 100 db stimulation intensity —— isotonic saline group — — Neo₃ group, – methylneomycl group.

F g. 76. Comparison between the 15 ears of the isotonic saline group d those of the Neo₂ (neomycin sulphat 2 g/kg 10 ears) and Neo₂ ₙ (neomyci sulphat and Vit. B—10 ears) groups t 100 db stim lation i tensit —— isotonic saline group — — — Neo₂ group, — — Neo₂ ₙ group.



F 77 Diagram of the destruction of the cochlear hai cells, plotted i black, in si ears f guinea pigs treated w th daily dose of 100 mg/kg body weight f eomycin sulphat for 30 days.

were seen again in the second half of the second coil These latter often remained incomplete up to the apex. The diagram of the missing sensory cells of the six examined guinea pigs is represented in Fig 77

The average distance over which all external hair cells are affected is 10 1 mm, whereas the average distance to the point where the external hair cells are fully present again is 16 mm (Table 52)

In all the animals the stria vascularis is severely affected, to the same extent as the destruction of the external hair cells. In the basal coil we often find only a strongly hyperchromic strip with rents.

Especially in the ganglia corresponding to the basal coil there is up to 75% destruction of the cells.

TABLE 52 *Distances are given along which all the external hair cells have disappeared and the distances to the point where all the external hair cells reappear in the ears examined*

The erage val es re calculated.

Guinea pigs, Group Neo_3	Absence of the three E.H.C. (mm)	Presence of the three E.H.C. (mm)
19 R	10 2	18.1
20 1	8 5	14
21 L	9.3	11.8
38 R	17 1	17 1
39 1	6	18
40 L	0.9	14
A erage distance	10 1	16

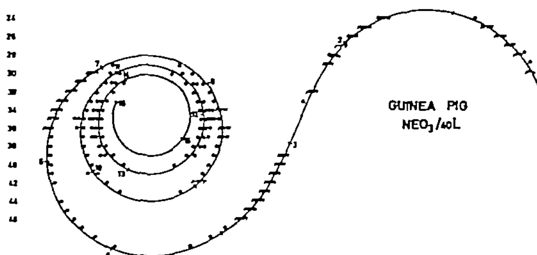


Fig. 78. Diagram of the cochlear spiral of the guinea pig Neo3 40 L with the remaining sensory cells. For the sake of clearness of the curve the cells still present have been plotted only on the distance of impairment.

The nerve fibres in the lamina spiralis ossea of the basal coil have markedly diminished in number. One animal presents again the peculiar severe impairment of the ganglion cells, most pronounced at the apex. This will be discussed later.

The nuclei of the limbus are impaired in 8 out of the 10 examined ears. Sometimes these nuclei have disappeared to the extent of 100%. This severe lesion is most pronounced at the limbus corresponding to the organ of Corti between 3 and 6 mm.

The surroundings. In several ears we established a decrease of the number of nuclei situated in the bony wall of the cochlea, especially in the wall that lies freely in the bulla. This decrease is generally more obvious in the basal coil and in the second coil.

The cochlear diagrams of guinea pig Neo3/40 L are fully represented in Figs. 78 and 79.

B. *Vestibular system*

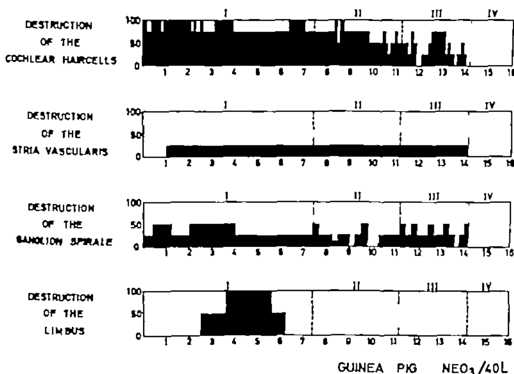
The functional tests have not been carried out on this group.

Histologically the vestibular systems in three animals are completely normal. In guinea pig 40 (two examined ears) the macula utriculi is slightly affected; the cristae ampullares and the macula sacculi are normal.

Group Methylneo3

Guinea pigs treated with a daily dose of 100 mg/kg body weight of methylneomycin for 30 days.

In this group no drop in the microphonics was established. 13 ears belonging to 8 animals were examined histologically.



F g. 79 Diagrams f the destructions of the sensory cells, of the stria vascularis, of the ganglion cells and f the limbus with guinea pig Neo_3/10 L.

A *Cochlea*

Not a single histological anomaly was established, neither in the organ of Corti nor in the stria vascularis and the ganglion spirale, nor in the limbus and the surroundings

B *Vestibular system*

In the vestibular system not a single anomaly was found either

GENERAL DISCUSSION AND CONCLUSIONS

Problem I

A first aim of this double study was tracing the location of the damage in the whole of the inner ear and establishing the chronology of its origin.

The damage in the cochlea is to be found in the organ of Corti the stria vascularis, the nerve fibres and the ganglion spirale the limbus and the cochlear wall

The damage to the limbus and the cochlear wall is a dilatory phenomenon, which only occurs with heavy intoxication of the cochlea.

The damage to the ganglion spirale and the nerve fibres in the lamina spiralis ossea is clearly secondary to the intoxication of the sensory cells. Rüedi established that the greater the disturbances in the organ of Corti the greater was the damage in the ganglion After examination of our series of animals it does not seem that one phenomenon is the result of the other but the impairment of the ganglion cells as well as the further disorganisation of the structure of the organ of Corti are two phenomena of deterioration that develop simultaneously

The damage in the sensory cells is an early and important phenomenon It begins in the basal coil and spreads in the direction of the apex. The external hair cells, as we have shown earlier are the first to be affected and of these the most medial is earliest

The latter phenomenon although already clearly visible in the basal coil is more obvious still in the more apical coils. These findings, established through histological study of the traditional cochlea slides by means of light microscopy confirm the findings of Kohonen, who studied the phenomena with phase contrast microscopy The internal hair cells are only affected later sometimes even after the partial collapse of the tunnel structure Although from the various diagrams and graphs, we may assume that the impairment starts in the basal coil and extends towards the apex, we also see that the damage to the internal sensory cells follows a less distinct pattern

In three animals we found a different pattern of intoxication. One animal whose two ears were examined histologically belongs to the group Neo$_4$ (daily dose of 100 mg/kg body weight of neomycin for 30 days) The second belongs to the group N (daily dose of 200 mg/kg body weight of kanamycin and of 15 mg/kg body weight of nialamide for 30 days) The third animal belongs to the group V$_2$ (total dose of 15 g/kg body weight of viomycin) In these three animals the internal hair cells were missing in

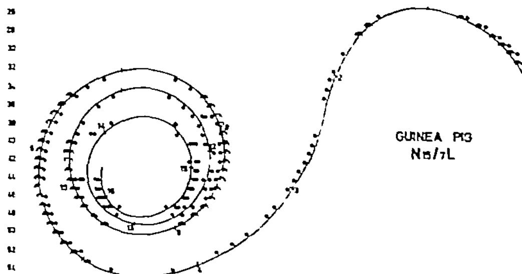


FIG. 80. Diagram of the cochlea spiral of the guinea pig N_b/7 L with the present sensory cells. Loss of the internal hair cells in the fourth coil, loss of the external hair cells in the lower coils, where the internal cells are still present.

the fourth coil, although the external hair cells had only incompletely disappeared in the fourth and the third coil (guinea pig Neo$_2$/38) or even lower and although the internal hair cells did show up in the lower coils. Consequently the impairment of the internal cells develops here from the apex towards the basal coil (Figs. 80 and 81).

The destruction of the external hair cells proceeds as already described from the basal coil to the apex.

The impairment of the ganglia is very pronounced in these three animals and seems more severe at the apex than at the base although this may be due to the normally less dense cell population of the apical ganglia. The ganglia are more affected than might be expected from the condition of the organ of Corti in that area. We might almost be led to think of a primary impairment of the ganglion cells, beginning in the apex, or of a reduction of the number of ganglion cells directly depending on the condition of the internal hair cells (Figs 82 and 83).

Since among all the examined ears (about 80) we only found three with this unusual pattern of intoxication—viz. affection of the external hair cell diminishing from basal coil towards apex, affection of the internal hai cells diminishing from apex towards basal coil and very severe affec t on of the ganglion cell apparently more at the apex—we concluded that ther was some abnormal susceptibility of these particular animals to the product, the doses given being too toxic or too high for these particular animals. From Kohonen s results there is a similar pattern of intoxication of the internal hair cells, diminishing from the apex towards the basal coil le reg rd th s as the normal pattern of intoxication. We may wonder

The innervation of the external cells is more dense in the basal coil than in the apical one (Smith, 1961 diagrams A and B Kohonen-Engstrom, 1965 according to gradients in radial and apical sense)

From these findings we may infer that the basal coil is the most important one because of its length, the number of cells, its metabolism and its innervation This great nerve density in the cells of the basal coil allows the supposition that these cells are either more active and consequently have a greater metabolism, or have a more delicate function than the cells in the higher coils.

Müsebeck (1964) established that following intoxication of the inner ear with streptomycin the first histochemical changes appeared in the stria vascularis

Neomycin kanamycin and streptomycin are carried to the peri and endolymph through the blood stream and are eliminated from the internal ear more slowly than from the blood Consequently the level of ototoxic antibiotics remains high for a longer time in the internal ear than in the blood (Voldrich 1965 Stupp & Rauch, 1965) With neomycin the elimination may take up to 55 hours, so that with daily injections a cumulative effect is obtained. This slow elimination is due to the slow resorption in the stria vascularis, and it is in connection with the poor resorbability peculiar to these antibiotics in general according to Stupp & Rauch (1965) who also state that all ordinary cells would be affected by such prolonged concentrations of toxic product.

Although all cells are affected in the internal ear eventually the external hair cells are affected first, and the resulting lesion follows a constant pattern So, besides the factor of intoxication there must be a second factor causing the cells to be affected in a certain definite order This second factor in our opinion is either the more delicate function or the greater activity of the cells, because the fact that the most intoxicated cells are exactly those with the greatest nerve density (Kohonen, 1965 our own tests)

Darrouzet and coworkers proved that with guinea pigs there is greater liability to acoustic traumata after intoxication by ototoxic antibiotics (Darrouzet 1963) This means that there is a clear connection between the greater sensitivity of the hair cell to the ototoxic antibiotics and a greater activity

Hypothesis of the process of intoxication as we drew it up after the study of other research work and our own tests

The ototoxic product is carried by the blood stream to the peri and endolymph where it is retained longer than in the blood because of the slow elimination by the stria vascularis. The resorption of ototoxic material is greatest in the stria vascularis of the basal coil The toxic effect makes itself felt slowly in the stria of the basal coil In consequence of this the resorption diminishes, the process of elimination lasts longer and the structures of the internal ear are exposed to the toxic product for a longer

time The sensory cells that are most important, be it because of their greater activity or because of their more delicate function, are affected first, viz. the external hair cells, beginning with the most medial and extending towards the more lateral ones, and from the basal towards the apical areas. With further intoxication more of the stria vascularis is affected, with reduced resorption, prolonged duration of elimination and longer exposure to the toxic product Thus more sensory cells are affected, and following repeated and prolonged exposure less sensitive cells are affected as well. After the disappearance of the sensory cell the whole structure of the organ of Corti collapses slowly and the corresponding nerve fibres and ganglion cells degenerate

PROBLEM II

A second purpose of this double study was the comparison of the toxicity of the different ototoxic antibiotics.

After studying the curves obtained in measuring the microphonics at the round window (Figs. 41 42, 43, 63, 66, 74 [illegible] and [illegible]) we may classify neomycin as most toxic antibiotic followed by kanamycin, viomycin, streptomycin and finally capreomycin in diminishing order of toxicity the last three of these antibiotics, however being much less toxic than neomycin and kanamycin. A comparison of the damage to the external hair cells (E. H C.) by the different drugs, based on histological examination and expressed in millimeters is shown in Table 33. Neomycin is the most ototoxic antibiotic then kanamycin, viomycin, capreomycin and finally streptomycin

TABLE 33 *Classification of the products according to the average distances on which all the external hair cells have disappeared and to the point where all the external hair cells are present again (this last distance is especially important)*

Product	Absence of the three E.H.C. (mm)	Presence of the three E.H.C. (mm)
Neomycin Neo[illegible]	10.1	16
Kanamycin		
[illegible]	6.33	—
[illegible]	3	[illegible]
[illegible]	4.73	11
[illegible]	3.93	[illegible]
P	1	[illegible]
[illegible]	—	[illegible]
[illegible]	0.9	[illegible]
Viomycin	1.1	[illegible]
[illegible]	0.16	0.23
[illegible]	—	—
[illegible]	—	—

mycin. If however we take into account the fact that when the streptomycin injections were administered on the same schedule as the viomycin and capreomycin, it had to be stopped because of its severe general toxicity and further that streptomycin affects the vestibular system whereas capreomycin does not cause any damage to this system we may conclude that streptomycin is more toxic than capreomycin and that capreomycin is the least ototoxic antibiotic of all the drugs tested.

Problem III

A third aim of this double study was to test the ability of certain drugs to reduce the toxicity of a few antibiotics.

Four groups of tests were carried out for this purpose and they will be discussed here

Group I

This comprises the series of animals K_8, N_{15}, N_4 and N_{16} to which nialamide was administered in subcutaneous daily injections of 1.5 mg/kg, 8 mg/kg and 15 mg/kg body weight respectively at the same time as kanamycin sulphate but with different syringes and needles and at different sites of injection. A daily dose of 200 mg/kg body weight of kanamycin sulphate was administered for 30 days. Looking through Fig. 42 and Tables 7, 8, 9 and 10 we see clearly that nialamide does not protect the cochlea of the guinea pig against the toxicity of kanamycin but gives protection against the slight acute general toxicity the slight loss of hair noticed in series K_8 was not observed in N_{15} and N_4. On the other hand there was a distinct impairment of the vestibular system in series N_{15}, which was not found with the series K and N_4

Group II

This comprises the series of animals K_2, P_1, P_2 and P_3, which were injected with a daily dose of 250 mg/kg body weight of kanamycin sulphate kanamycin monopantothenate kanamycin dipantothenate and kanamycin tripantothenate for 20 days.

From the curves obtained in Fig. 43 and from Table 10, we deduce that kanamycin monopantothenate is clearly less toxic than the sulphate the dipantothenate and the tripantothenate.

That in group II much more disturbance of the vestibular system occurred than in group I, is perhaps due to the fact that the kanamycin was obtained from two different laboratories. These two kanamycins apparently differed in their basic composition and contained different quantities of kanamycin A and B

Group III

This comprises the series of animals Neo_3 and $Methylneo_3$, which were injected subcutaneously with a daily dose of 100 mg/kg body weight of neomycin and methylneomycin for 30 days.

Electrophysiological and histological examination revealed no toxicity with this modified neomycin. But methylneomycin has lost all antibiotic activity

We can imagine how selective the ototoxicity must be since a slight modification in the formula makes all toxicity disappear

The question is whether and how far the bactericidal capacity and the ototoxicity are based on one and the same activity of the product It would be interesting to examine the percentage of methylneomycin in the endolymph and the perilymph, and the rate of elimination from the internal ear in comparison to the research carried out with neomycin and kanamycin (Voldrich, 1965 Stupp & Rauch, 1965)

Group IV

This comprises the series of animals Neo- and Neo$_{3}$ $_{B}$. A mixture of Vit B_1 B_6 and B_{12} was daily administered together with neomycin (daily dose of 200 mg/kg body weight) for 10 days.

Electrophysiological examination (Fig 76) did not reveal any protective action of these vitamins, administered in this form Hence no histological research was carried out

Problem IV

A fourth aim of this double study was to establish a possible connection between a drop in the microphonics and an accurate location of the damage

Both examinations carried out on a series of animals develop in a fully parallel way and confirm each other The comparison between the two methods of investigation done on one individual animal was disappointing and did not allow any conclusions. After all, this is easily understood when one thinks of the defects and the limitations of both methods The electrophysiological test is a dynamic test, but since the electrode is placed at the round window the information obtained almost exclusively concerns the basal coil

The histological examination gives us a survey of the whole cochlea, but does not teach us anything about the function of the cell We only state whether a cell is apparently normal or apparently affected or whether it is absent

SUMMARY

An electrophysiological and histological study was carried out with series of guinea pigs that were previously treated with different streptomyces antibiotics

Purpose of the experiment

1 Finding the locations of the lesions caused by ototoxic antibiotics in the inner ear and the chronology of their appearance

2. Comparison of the toxicity of the antibiotics used

3 Testing of certain drugs with a view to a reduction of the ototoxicity of certain antibiotics.

4 Establishing any possible relation between a decrease in cochlear microphonics and an accurate location of the lesions.

The electrophysiological study the technique of which has been described before (*Acta Oto-laryng* (Stockh) 1962, Suppl *171*) consisted in the registering of the cochlear microphonics on series of 15 ears. The histological study of a minimum of 5 inner ears in each series was carried out by means of the usual light microscopy The cochlea was graphically re constructed according to Guild (1921) and Schuknecht (1953)

The histological lesions caused by the ototoxic antibiotics in the organ of Corti in the stria vascularis, in the ganglion spirale in the limbus, in the surrounding tissues and in the different vestibular structures were de scribed with great care

A first series of experiments was carried out with kanamycin Simulta neous injections of kanamycin and nialamide did not diminish the ototoxi city of kanamycin either from an electrophysiological or histological point of view A slight decrease of the acute toxicity was found On the other hand kanamycin monopantothenate diminished obviously the ototoxicity of kanamycin in the guinea pig both electrophysiologically and histologi cally Kanamycin dipantothenate and tripantothenate appeared somewhat less toxic than kanamycin sulphate.

A second series of experiments was carried out with streptomycin, vio mycin and capreomycin Here the acute toxicity of streptomycin was rather important and the chronic toxicity for the vestibular system obvious. Capreomycin showed strikingly little toxicity for the inner ear of guinea pigs (electrophysiological and histological tests)

A third series of experiments was carried out with neomycin and methyl neomycin The important ototoxicity of neomycin established electrophy siologically and histologically in the guinea pig was not found again with

methylneomycin. On the other hand, the simultaneous injections of neomycin and vitamin B did not diminish the ototoxicity of neomycin in the guinea pig

After examination of previous research and of our own experiments, a hypothesis about the mechanism of intoxication of the inner ear by streptomyces antibiotics is proposed. We have stressed the fact that the ototoxic antibiotic stays in the inner ear fluids longer than in the blood because of the slower elimination and that among the cochlear cells the more active ones appeared to be more sensitive to the ototoxic antibiotic

RÉSUMÉ

Une étude électrophysiologique et histologique a été effectuée sur des groupes de cobayes traités avec différents antibiotiques streptomyciniques. Cette double étude avait pour but:

1° établir la localisation et l'ordre chronologique des lésions, causées par les antibiotiques ototoxiques à hauteur de l'oreille interne;
2° comparer la toxicité des antibiotiques utilisés;
3° contrôler l'action de certains produits ou vitamines diminuant éventuellement l'ototoxicité de ces antibiotiques;
4° rechercher une relation entre une diminution de l'effet microphonique et la localisation exacte des lésions dans l'oreille interne.

L'étude électrophysiologique, dont la technique a été décrite antérieurement (*Acta Oto-laryng.* (Stockh.) 1962 Suppl. 171), consistait à mesurer l'effet microphonique par groupes de 15 oreilles. L'étude histologique d'un minimum de 5 oreilles par groupe a été effectuée par microscopie simple. La cochlée a été reproduite en graphique selon Guild (1921) et Schuknecht (1953).

Des lésions histologiques, causées par les antibiotiques ototoxiques, ont été décrites dans l'organe de Corti, la strie vasculaire, le ganglion spiral, le limbus, les tissus environnants et dans les structures vestibulaires.

Une première série d'expériences a été effectuée avec la kanamycine. L'injection combinée de kanamycine et de nialamide ne diminue pas l'ototoxicité de la kanamycine ni au point de vue électrophysiologique ni au point de vue histologique. Une légère diminution de la toxicité aiguë a été constatée. Par contre le monopantothénate de kanamycine réduit manifestement l'ototoxicité de la kanamycine chez les cobayes (étude électrophysiologique et histologique). Le dipantothénate et tripantothénate de kanamycine semblent être légèrement moins toxique pour l'oreille interne du cobaye que le sulphate de kanamycine.

Une deuxième série d'expériences a été pratiquée avec la streptomycine, la viomycine et la capréomycine. La toxicité aiguë de la streptomycine est assez importante de même que la toxicité chronique pour le système vestibulaire. La capréomycine s'est révélée très peu toxique pour l'oreille interne du cobaye sur le plan électrophysiologique et histologique.

La troisième série d'expériences s'est effectuée avec la néomycine et la méthylnéomycine. L'ototoxicité importante de la néomycine, constatée électrophysiologiquement et histologiquement chez les cobayes, contraste avec l'absence d'ototoxicité de la méthylnéomycine. Par contre un traite-

ment de néomycine associée à une certaine dose de vitamine B ne diminue pas l'ototoxicité de la néomycine

Après étude des publications antérieures et de nos propres expériences, nous proposons une hypothèse concernant le mécanisme d'intoxication de l'oreille interne par les antibiotiques streptomyciniques. L'antibiotique ototoxique resterait plus longtemps dans les liquides labyrinthiques que dans le sang suite à son élimination plus lente. Les cellules cochléaires les plus actives seraient également plus sensibles à l'influence nocive des antibiotiques ototoxiques.

ZUSAMMENFASSUNG

Eine elektrophysiologische und histologische Untersuchung ist durchgeführt worden an Meerschweinchengruppen die mit verschiedenen Streptomyces-Antibiotika behandelt worden waren

Dieser Doppelversuch hatte zum Zweck

1 die Lokalisierung und die chronologische Reihenfolge der durch ototoxisch wirkende Antibiotika erzeugten Läsionen im Innenohr festzustellen

2 die Toxizität der verwendeten Antibiotika zu vergleichen

3 die Wirkung bestimmter Produkte bzw Vitamine die evtl die Ototoxizität dieser Antibiotika vermindern zu prüfen

4 einen Zusammenhang zwischen einer Verminderung des Mikrophoneffekts und der genauen Lokalisation der Läsionen im Innenohr zu suchen

Der elektrophysiologische Versuch, dessen Technik schon früher beschrieben worden ist (*Acta Oto-laryng* (Stockh) 1962 Suppl 171) bestand in der Messung des Mikrophoneffekts in Gruppen zu 15 Ohren Die histologische Untersuchung bei einem Minimum von 5 Ohren pro Gruppe wurde mittels einfacher Mikroskopie durchgeführt Die Cochlea ist graphisch nach Guild (1921) und Schuknecht (1953) dargestellt worden

Histologische Läsionen, durch ototoxische Antibiotika bedingt sind im Cortischen Organ, der Stria vascularis, dem Ganglion spirale, dem Limbus, den umgebenden Geweben und den Vestibularisstrukturen beschrieben worden

Die erste Untersuchungsserie ist mit Kanamycin durchgeführt worden Die Injektion der Kombination von Kanamycin mit Nialamid zeigt weder elektrophysiologisch noch histologisch eine Verminderung der Ototoxizität des Kanamycins Eine leichte Verminderung der akuten Toxizität wurde festgestellt Im Gegensatz hierzu ist die Toxizität von Kanamycin Monopantothenat deutlich vermindert (elektrophysiologische und histologische Untersuchungen) Di und Tripantothenat scheinen eine leicht geringere Toxizität auf das Innenohr des Meerschweinchens auszuüben als Kanamycin-Sulfat

Eine zweite Untersuchungsserie ist mit Streptomycin, Viomycin und Capreomycin durchgeführt worden Die akute Toxizität des Streptomycins ist beachtlich, ebenso die chronische Toxizität auf das Vestibularsystem Capreomycin erwies sich bei der elektrophysiologischen und histologischen Untersuchung als wenig toxisch auf das Innenohr des Meerschweinchens.

Die dritte Untersuchungsserie ist mit Neomycin und Methylneomycin durchgeführt worden. Die ausgeprägte Ototoxizität des Neomycins, die am

Meerschweinchen elektrophysiologisch und histologisch festgestellt wurde steht im Kontrast zum Fehlen der Ototoxizität bei Methylneomycin. Die Kombination von Neomycin mit Vitamin B in einer gewissen Dosis vermindert die Ototoxizität dieses Antibiotikums nicht

Nach Studium der früheren Publikationen und unserer eigenen Untersuchungen schlagen wir eine Hypothese vor über den Mechanismus der Intoxikation des Innenohres durch die Streptomyces-Antibiotika das ototoxisch wirkende Antibiotikum würde aufgrund seiner langsameren Ausscheidung in der Labyrinthflüssigkeit eine längere Verweildauer haben als im Blut Die cochlearen Zellen, die eine höhere Aktivität leisten, dürften auch die höchste Empfindlichkeit auf den schädlichen Einfluss der ototoxisch wirkenden Antibiotika zeigen

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THE LABYRINTHINE CAPSULE NORMAL STRUCTURE AND PATHOGENESIS OF OTOSCLEROSIS

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THE LABYRINTHINE CAPSULE NORMAL STRUCTURE AND PATHOGENESIS OF OTOSCLEROSIS

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CONTENTS

The structure of the labyrinthine capsule has been an enigma with many unusual features, all apparently unrelated to each other so that no comprehensive understanding of this bone as an entity with interrelated physiological mechanisms has seemed possible. The histologic structure of the labyrinthine capsule is usually considered to be normal unless otosclerotic bone is present replacing portions of the original capsule. In-between stages of abnormality are not recognized. The middle layer of the labyrinthine capsule is described in the literature as consisting of endochondral bone which once formed, persists throughout life without change. The presence of true cartilage foci is denied by some while others describe their presence as abnormal. Peculiar blood vessels are described which have unusual staining characteristics of their walls. Unusual blue-staining bone is sometimes seen about blood vessels in apparently normal specimens, as well as in otosclerotic specimens. An area of predilection for otosclerotic involvement is definitely agreed upon within and about the fissula ante fenestram. However otosclerotic foci also develop within the capsule with no relation to this area of predilection. It is not clear what is normal and what is abnormal within this complicated structure.

Before pathological processes can be adequately recognized and their pathogenesis determined, it is essential that there be a clear recognition of the normal processes occurring throughout the life of a tissue. Only then can deviations from the normal be recognized and explained.

The present study presents an overall histological picture of the human labyrinthine capsule from birth to old age demonstrating the interrelations between cartilage, bone, blood vessels and soft tissue. Certain changes will be described which occur normally in all temporal bones. An attempt to relate the abnormal progression of certain of these changes to the development of otosclerosis will be made.

Methods and Materials

Human temporal bones from 27 patients (48 specimens) were studied ranging in age from birth to 82 years of age. Thirteen of the patients were males, from one day to 79 years of age and fourteen of the patients were females, ranging in age from 15 minutes to 82 years of age. In 46 of the specimens, the appearance of the labyrinthine capsule was consistent with what has generally been considered to be normal. Two of the specimens contained typical otosclerotic foci involving the stapediovestibular joint with ankylosis, and were from a 40-year old white female. Four specimens were from Negro males, one pair from a one-day old baby and the other pair from a 48-year old male.

The temporal bones were vacuum fixed in 10% neutral buffered formalin demineralized by chelation with a 0 7M solution of the tetrasodium salt of EDTA at pH 7 4 and 37 C (Gussen and Donahue 1965) and vacuum embedded in parlodion (Donahue and Gussen 1966) Sections were cut at 20 micra and were stained with hematoxylin and eosin

Mayer (1917) Bast (1930 1942) Richany Anson and Bast (1960) and many others have divided the labyrinthine capsule into three more or less clearly defined layers of bone The inner endosteal layer has been described as being always thin and fairly uniform The middle layer is described as consisting of immature endochondral bone which, once formed, persists as such throughout life This layer also contains globuli ossei with cartilage matrix remnants which are usually referred to as calcified matrix remnants. Cartilage foci are also described within this layer most commonly in relation to the basal and middle turns of the cochlea. The outer layer is referred to as the periosteal layer and is described as an investing layer for the middle so-called endochondral layer

Bast (1930 1942) and Richany Anson and Bast (1960) have described in detail the fetal development of the cartilaginous and bony otic capsule The bone is preceded by a cartilaginous model with successive ossification centers appearing at specific locations within the capsule At about 19 to 21 weeks, the middle layer of the fetal otic capsule contains globuli ossei with interglobular cartilage matrix (referred to as intrachondrial bone by Richany Anson and Bast) which are separated by large areas of bone marrow and loose connective tissue where the cartilage of the original capsule had been completely excavated. The authors then describe replacement bone filling in these marrow spaces between the globuli ossei, gradually causing this middle layer to become more solid During the last week of fetal life and immediately after birth, the bone marrow is more rapidly replaced by bone Richany Anson and Bast refer to this replacement bone which forms the bulk of the middle layer as endochondral bone. They describe the formation of osteoblasts within the marrow and connective tissue of the excavated areas and consider this connective tissue a form of periosteum the osteoblasts then depositing bone upon the surfaces of the globuli ossei, and gradually filling in the excavated areas

In the present study four newborn specimens were available one pair from a 16-minute old female and the other pair from a one-day old male These specimens revealed a clear-cut demarcation of three layers of the otic capsule (Fig. 1 A) The photograph demonstrates the entire thickness of the labyrinthine capsule adjacent to the lowermost portion of the cochlear basal coil. Considerable replacement bone fills in the areas between the foci of globuli ossei in certain areas, while definite marrow areas are still present undergoing replacement by bone It is interesting to note the similarity in appearance of the periosteal and subperiosteal bone layer which

is quite thick in this area to the replacement bone surrounding and encasing the foci of globuli ossei In Fig 1 B we see a high power view of the same area revealing osteoblasts lined up along the surfaces of the replacement bone which has already formed all of which encases the foci of globuli ossei No cartilage is present

What is represented here is the formation of endosteal membranous bone not endochondral bone filling in the previously excavated areas. Endochondral bone is bone that has formed in cartilage the degenerated cartilage being replaced bit by bit by osteoblasts that have invaded the cartilage model This is not what we see here True the area originally was represented by cartilage but that cartilage degenerated and was completely replaced by cellular marrow and connective tissue similar to the medullary cavity of long bones. This marrow and connective tissue then becomes filled in and replaced by bone The bony surfaces lining the marrow spaces are endosteal surfaces, so that the osteoblasts can be considered to be lined up alongside the endosteal surface of bone surrounding the marrow and forming endosteal membrane bone which gradually fills in the marrow spaces. The marrow spaces remain as the blood vessel canals The globuli ossei, however have formed as endochondral bone (bit by bit replacement of degenerated cartilage) and are embedded in the greater bulk of endosteal membrane bone Enlow (1963) refers to this process, in general as the compaction of spongy bone and describes the typical endosteal bone that forms as "convoluted" bone forming as irregular sinuous convolutions of bone which are easily recognized in microscopic sections. As the cancellous bone is filled in, or compacted, remnants of endochondral spicules become incorporated into the new bone Layers of lamellae produced by endosteal growth but which lack the irregular sinuous convolutions characteristic of compacted endosteal bone are termed the inner or endosteal circumferential lamellae This latter type of endosteal lamellae forms the *inner lining bone of the labyrinthine capsule Blood vessel canals that be*come incorporated within endosteal inner circumferential lamellae are characteristically oriented in a radial manner perpendicular to the principal axis Their radial pattern is in contrast to the predominantly longitudinal arrangement of canals in periosteal circumferential bone Canals included within endosteal compacted bone are typically arranged in an irregular tortuous manner which corresponds to the convoluted organization of the bone substance itself A discussion of the labyrinthine capsule canal system containing blood vessels will be discussed in greater detail later

*The labyrinthine capsule is thus seen to consist almost entirely of mem*brane bone with the scattered foci of globuli ossei representing endochondral bone The periosteal layer when present as outer circumferential lamellae is easily identified However the demarcation between sub-periosteal bone and endosteal convoluted bone is not always clear The author will refer to the labyrinthine capsule as consisting of an inner circumferential endosteal layer a middle endosteal convoluted layer containing scattered endo-

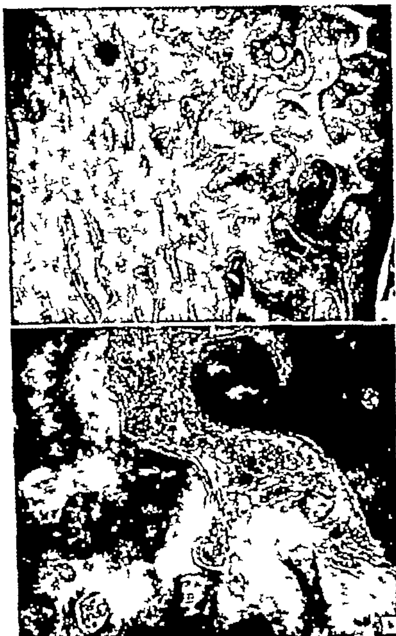


FIG. 1 (A) Labyrinthine capsule of newborn female (15 minutes) djacent t basal coil of cochlea. Not inner endosteal layer (right lower field). The middle layer contains bone marrow and globuli ossei, portions of which re embedded i endosteal membrane bone forming by pposition. Note similarity of the endosteal bone filling i the marrow t the subperiosteal membrane bone. H&E 35.—(B) Higher power iew demonstrating formation of endosteal membrane bone bout the globuli ossei, filling in the bone marrow. Not osteoblast lined up long the bone surfaces forming bone by pposition, nd the bsence of cartilage. H&E 125.

chondral globuli ossei and an irregular outer periosteal layer which for the most part is poorly demarcated from the endosteal convoluted bone save where it forms periosteal circumferential lamellae

To recapitulate The labyrinthine capsule is preformed in cartilage. The cartilage calcifies and degenerates, and part of it is replaced by bone (endochondral globuli ossei) However most of the cartilage is completely replaced by bone marrow and loose connective tissue Osteoblasts arise within this soft tissue and deposit membrane bone by apposition on the endosteal surfaces of the globuli ossei bordering the marrow This endosteal convoluted membrane bone gradually fills in the bulk of the middle layer encasing the spicules of endochondral globuli ossei, and is continuous with the membrane endosteal bone of the inner layer and with the membrane periosteal bone of the outer layer The labyrinthine capsule therefore is essentially a membrane bone with globuli ossei representing scattered foci of endochondral bone within its inner and midportions

GENERAL FEATURES OF ENDOSTEAL AND PERIOSTEAL MEMBRANE BONE

Membrane bone is formed by mesenchymal cells which transform into osteoblasts and secrete the essential matrix There is no bit by bit replacement of calcified, degenerating cartilage by bone Endosteal and periosteal bone deposition throughout life represent membrane bone formation.

It has long been recognized that matrix forming cells have the ability to produce bone or cartilage (or fibrous tissue) depending to a great extent on the local environment of these cells. For example, Ham (1930) was the first to recognize that cartilage formation, rather than bone formation might occur in healing fractures if the oxygen tension was decreased. Fell (1932) demonstrated that cultures of endosteal cells produced both bone and cartilage Endosteal cells (like periosteal cells) form membrane bone and do not normally pass through a cartilage stage yet these cells were shown to have the capacity to form cartilage in an environment conducive to its formation

More recent studies by Bassett (1964) and by Shaw and Bassett (1967) have shown that not only can an inadequate supply of oxygen produce chondrogenesis by cells usually forming bone but that variations in the concentration of oxygen produced varying results These authors subjected cultures of embryonic bone to varying concentrations of oxygen, and demonstrated that with intermediate oxygen concentrations, there was degeneration of chondrocytes, as well as transformation of chondrocytes directly into osteoblasts.

Gardner (1956) refers to this cartilage formation occurring in membrane bones as secondary cartilage formation The secondary cartilage develops after ossification begins and consists of large vesicular cells with relatively little intercellular matrix *It takes part in the growth processes, but is not* part of the cartilage primordium Examples of membrane bones with secondary cartilage growth centers are the mandible the clavicle and the *suture areas of the bones of the skull vault*

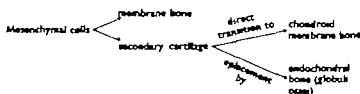
DeBeer (1937) found secondary cartilage to be commonly associated with membrane bones which are subjected to "precocious strains and stresses *He suggested that periosteal cells are capable of forming nodules of* secondary cartilage He also considered secondary cartilage similar to the cartilaginous tissue appearing in callus formation and sesamoid bones

There has been some confusion in terminology For example Moss (1961) describes secondary cartilage in fish bone but refers to it as h ondroid The author herself (Gussen in press) has reffered to this type

of cartilage as chondroid cartilage. The transformation of this type of cartilage to bone may follow the usual processes of endochondral ossification, or may occur by direct transformation of the cartilage matrix to bone matrix without breakdown and resorption of the cartilage. Secondary cartilage has been described by Moss (1958) in the frontal suture area of the rat and in the sagittal suture area of the rat by Pritchard *et al* (1956) They describe areas of transformation of secondary cartilage directly into bone without prior resorption, as well as areas of bit by bit bone replacement of the degenerated cartilage

Enlow (1962) refers to secondary cartilage as chondroid and its direct transformation to bone as chondroid bone formation He describes chondroid bone as being normally present at the crest of bone tubercles and bony processes (membrane bone areas) in rapidly growing bones, and believes it to be similar to the tissue on the growing alveolar crests surrounding teeth Enlow believes that chondroid bone provides anchorage and perhaps resistance to pressure He states that although the fibrous matrix in bony processes is subject to tensile forces, the individual chondroid cells are resistant to the pressure exerted on them by surrounding fibrous matrix

To recapitulate Membrane bone has the ability in certain areas and under certain conditions to form a cartilaginous type of tissue This cartilage is referred to as secondary cartilage, or chondroid cartilage or chondroid This secondary cartilage, formed within membrane bone mav become ossified It may do so in two ways it may degenerate and be replaced bit bv bit by osteoblasts, forming endochondral bone or it mav transform directly into bone, without degenerating or being resorbed, and is then referred to as chondroid bone Chondroid bone is, therefore membrane bone which, instead of being formed directly has gone through a stage of secondary cartilage formation, and has continued directly through this secondary cartilage stage to bone Here the original endosteal or periosteal mesenchymal cells become cartilage cells which then become bone cells. There is no loss of the cell with replacement by another cell in this process. Chondroid bone is a special form of membrane bone which has passed through a secondary cartilage stage. Membrane bone therefore may be formed directly by the mesenchymal cell or may first pass through a secondary cartilage (or chondroid cartilage) stage before becoming bone Secondary cartilage foci forming in membrane bone may also degenerate with bit by bit replacement by bone, forming endochondral bone



CARTILAGE FOCI AND GLOBULI OSSEI

Cartilage foci were found in the present study in every specimen examined. These cartilage foci contained cartilage cells and were seen in all specimens about the membranous cochlea and, rarely about the semicircular canals. They appeared to be least numerous at birth where the labyrinthine capsule was narrow Although some of the older temporal bones revealed decreased numbers of cartilage foci, other older specimens contained increased numbers, so that variation occurred from specimen to specimen, rather than with age The most prominent and consistently present cartilage was in the bony septum between the basal and middle turns of the cochlea, which sometimes occurred as an actual lining layer along portions of the upper basal and lower middle turns However cartilage foci were present in the bone about the entire cochlea, including the apical turn, to varying degrees in the different specimens. The cartilage foci were irregular in size and shape and consisted of very pale-staining somewhat distorted chondrocytes and small amounts of uncalcified, pale staining matrix.

Certain changes were seen within many of these cartilage foci which were similar in all the specimens of all ages (Fig. 2) Many of the chondrocytes were degenerating or had disappeared, and varying numbers of macrophages were noted within the involved cartilage lacunae The macrophages, themselves, often appeared to be undergoing degeneration The thin matrix bars between the degenerating, phagocytized cells were uncalcified and were breaking down. At times, a few calcified septa were seen, three or four at the most rather than any massive type of matrix calcification. Osteogenic vascular buds from the surrounding bone extended into the opened, excavated, "prepared cartilage lacunae forming bone The blood vessels giving rise to the osteogenic buds had deep basophilic staining of their walls They appeared to be "active" osteogenically in all the specimens supplying the osteogenic buds for endochondral bone replacement The new endochondral bone thus formed usually did not completely replace the cartilage focus, but only partially replaced it, forming islands of bone with uncalcified cartilage matrix remaining (globuli ossei) (Fig 3) This process of chondrocyte degeneration and phagocytosis by macrophages, with endochondral ossification forming globuli ossei, could be demonstrated in every temporal bone regardless of age or sex, and appeared to be a continuing process throughout life

As mentioned before the cartilage foci containing cartilage cells were seen throughout life These cartilage foci were within the endosteal inner

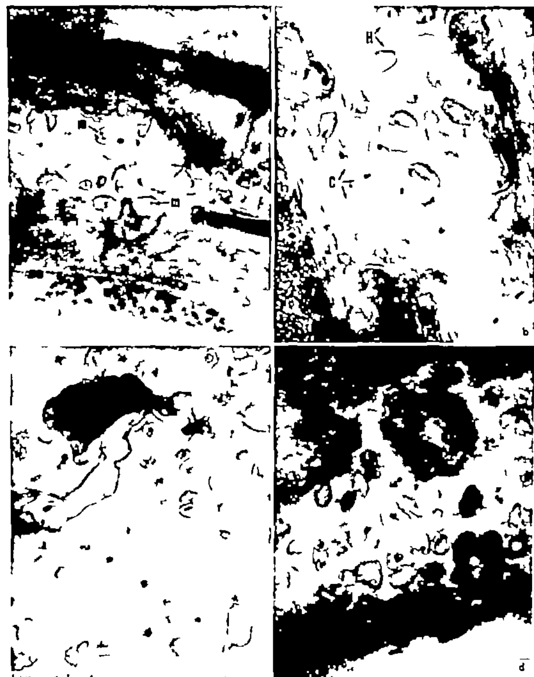


FIG. 2. Secondary rtilag foci derg l g repl cem t by doch ndral gl bull asel Reprod ced from The Am rica J rn l f Anat my (Gussen, In press) (A) Cartil ge focu adj ce t t middl coil of cochlea N t degenerated h ndrocytes (C) d occa ional macrophages (m) a w ll a proml ent blood vessel a d ndocho dral bo formati n. 31 year ld male H&F 280.—(B) Cartilage focu djacent t middl cochlea coil Not degen rated hondrocytes (C) d lcifleati f l divid l matrix w ll (B) with endochondral bone f rm ti 31 yea ld mal H&E 450.—(C) Cartil ge focu adjace t t basal coil f cochlea. Not degeneration f cartil g cell with ingrowth f osteogenic scula buds and doch dral bon formati I divid l cartil ge lacunar wall re calcified. P le-st l l g macrophages re present 64- old f m l H&E 450.—(D) Cartilage l septum between basal a d middle cochlear t rns. N t degenerati g ch ndrocytes with macrophages present i scattered lacu ae Endochondral bone f rm ti is proml t 79- ear ld mal H&E 450

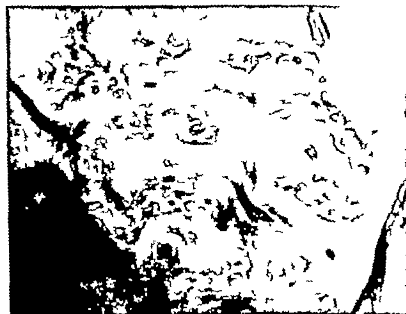


Fig. 3 Endochondral globuli ossei adjacent to basal cochlear turn with remnant lacunae of uncalcified cartilage matrix. Note deeply staining blood vessel walls. 8-year-old male H&E ×120 Reproduced from Amer J Anat. (Gussen, In press)

layer of bone, often bordering directly on the soft tissue lining of the labyrinth, or within the innermost portion of the middle labyrinthine capsule layer Streeter (1918) has described the unusual embryological development of the semicircular canal portion of the labyrinthine capsule with its constantly changing cartilaginous character for accommodation of the enlarging semicircular canals The new cartilage that forms and degenerates repeatedly during this process has been described by Streeter as arising from reticular tissue about the membranous canals. This ability of the soft tissue mesenchymal cells adjacent to the inner lining of the bony labyrinth to form cartilage is retained throughout life and it is from these areas that the secondary cartilage foci arise which represent growth foci throughout life It is not clear how long these cartilage foci remain as such before undergoing endochondral bone replacement. The globuli ossei thus formed during life are evidence that the growth area had once occupied that particular location.

In his demonstration of cartilage resorption in the fetus, Bast (1932) noted certain features which he considered peculiar to the region about the semicircular canals. He described the removal of degenerating chondrocytes by phagocytes, with the subsequent deposition of bone by osteoblasts There was just enough cartilage destruction to allow blood vessels to penetrate rather than any massive destruction of cartilage

The resorption of matrix by macrophages has also been described by

Goldhaber (1961) who induced bone resorption in tissue cultures of mouse calvariae and demonstrated by time-lapse films the presence of very motile macrophages attacking bone spicules and participating directly in the resorption process

Anderson and Parker (1966) in an electronmicroscopic study of endochondral bone formation in the femur of newborn rats, showed areas where macrophages preceded the capillary endothelium The cartilage matrix was calcified only for a distance of one to three chondrocyte capsules in advance of the invading capillary complex. Some matrix septa were very thin and did not actually calcify The degree of calcification of the matrix walls was very variable, only about one-third of the septa actually showing complete calcification.

In a preliminary study the author demonstrated the presence of particulate and fragmented bone canaliculi within the matrix of the interglobular spaces (Gussen 1967) At that time this was interpreted as evidence of breakdown of bone canaliculi and matrix, suggesting that the globuli ossei might represent foci of bone resorption This is an incorrect interpretation The particulate matter demonstrated in the previous study is now recognized as evidence of new endochondral bone formation with beginning formation of canaliculi by the osteoblast cell processes.

Once the cartilage foci are partially replaced by new endochondral bone, the resulting globuli ossei with their uncalcified cartilage remnants, remain as such throughout life. Manasse (1897) recognized the change from cartilage cells to bone cells of the globuli ossei, but considered the process one of metaplasia rather than the replacement of the cartilage cells by endochondral bone Shin Izi Ziba (1911) believed that changes did occur in these areas in later life and felt that the cartilage areas were smallest early in life Wittmaack (1919) also believed that these areas underwent change throughout life Mayer (1917) recognized that the cartilage cells degenerated ("dissolved") and were replaced by bone cells. However he did not realize that this process continued throughout life.

It is emphasized that at no time in the normal cartilage is any massive type of calcification of cartilage matrix seen neither in the original cartilage focus nor in the matrix of the interglobular spaces Many authors have stated that the cartilage matrix of the interglobular spaces is calcified matrix (Manasse 1897 Kosokabe 1922 Costa and Covell 1965 Richany Anson and Best 1960) Calcified cartilage matrix cannot maintain itself in the body and is resorbed following loss of its chondrocytes. In addition, calcified *cartilage matrix is readily identifiable, even in decalcified sections, by its* deeper affinity for hematoxylin and its more sharply accentuated lacunar margins There is no evidence of calcification normally in the cartilage matrix of the interglobular spaces or in the cartilage cell foci save for the occasional minimal calcification preceding endochondral bone formation as described.

As mentioned before membrane chondroid bone forms by direct transformation of secondary cartilage to bone It differs from endochondral bone in that the same mesenchymal cell that transformed into the secondary cartilage cell transforms directly into the osteoblast Similarly secondary cartilage matrix is incorporated directly into the bone matrix.

The soft tissue adjacent to the inner endosteal layer of the labyrinthine capsule (including the spiral ligament) contains mesenchymal cells which have both osteogenic and chondrogenic ability In many specimens, a continuous uncalcified cartilage lining could be demonstrated along portions of the bony cochlea (Fig 4 A) usually along the upper basal and lower middle cochlear turns This lining cartilage often contained degenerating chondrocytes or empty lacunae and in its deeper portion showed the processes of endochondral ossification with the resulting globuli ossei. The surface of the cartilage, however showed direct transition to bone forming chondroid membrane bone (Fig. 4 B) Small numbers of mesenchymal cells were seen along the cartilage and bone surfaces, appearing to enter into small cartilage and chondroid membrane bone defects The inner lining of the bony cochlea thus consists of chondroid membrane bone as well as areas of cartilage undergoing endochondral globuli ossei replacement or direct transformation to chondroid membrane bone

The endosteal surface of the semicircular canals consisted of membrane chondroid bone and, here too, occasional mesenchymal cells could be seen within the uneven surface of the lining bone

The inner endosteal circumferential lamellae lining the membranous labyrinth vary considerably in thickness from one location to another and from specimen to specimen Most specimens reveal layers of almost acellular chondroid membrane bone parallel to the surface These lining bone layers tend to be thickest in sharp curves or turns within the labyrinthine system or at the sites of the cribriform areas of nerve passage to sensory areas

Th bone of the modiolus and the bony spiral laminae appear virtually without lacunae Only where a larger amount of bone is present in some a s of the modiolus are occasional bone cells within lacunae seen As the cochlear and vestibular nerve branches leave the bony labyrinth to enter th nsory areas, they spread out through interdigitating spicules of bone which project from the inner surfaces of the chondroid membrane bone lining These interdigitating bone spicules are acellular and without lacunae and have the appearance in some areas of calcifying or ossifying cartilage matrix Mesenchymal cells are present about them and are occasionally



FIG. 4 (A) Sept m between basal nd middl t rns f cochl Not prominent li ing f secondary cartilage 69-yea old femal H&E 30 R prod ced from Amer J An t (Gussen, I press —(B) High pow iew Not degeneration f artil g cell nd do-ch ndral bone formati n i deeper portion. S rf ce cartil ge matri ha transf rmed directly t bon ppeari g rim f acellul chondroid m mbrane bone Note oc ca ional mesenchymal cell i rf ce depressions. 69-year ld femal H&E 280. Reproduced from Amer J A t. (G sen, I press)



Fig. 5 Cribriform plate in ampullary region of semicircular canal. Note interdigitating spicules of acellular bone with surrounding mesenchymal cells. Arrow indicates pale matrix, not yet ossified. Note pale staining, depolymerized inner endosteal layer of capsule at bottom. 62-year-old male. H&E 450.

"caught" between the criss-crossing spicules (Fig 5). Almost all specimens revealed formation of new bony spicules of this kind, resembling an in between type of matrix. The modiolus and the thin, bony spiral laminae consisted of these fragile acellular matrix spicules as well.

'REMODELING OF PERIVASCULAR BONE

It appears to be largely agreed that remodeling of the middle layer of the labyrinthine capsule does not occur Nager (1947) states that the middle layer remains in its embryonic state of development throughout life and that the vital bone processes of resorption and formation are absent.

Despite the rather universal agreement on the lack of new bone formation or of bone resorption there are occasional doubts For example Costa and Covell (1965) feel that it is erroneous to think of the middle layer of the otic capsule as incapable of a continuous remodeling process, but consider that such processes as might exist take place very slowly Engström and Röckert (1962) utilizing polarized light and soft roentgen microradiography described changes with age in the vascularity fibrillar texture and mineralization in the labyrinthine capsule and found evidence of small areas of replacement in the middle layer And yet when occasional changes were recognized they were always considered to be abnormal processes Weber (1933) for example in his beautiful description of bone changes about the blood vessels, considered these changes as preliminary stages of otosclerosis. Wolff and Bellucci (1964) and Wolff and Lempert (1965) describe changes in the perivascular bone but also believe it to be an abnormal process representing incipient otosclerosis. Aside from blue mantles, which all seem to agree represent new bone, no evidence of new bone formation is recognized nor are resorption cavities or osteoclasts seen in normal specimens

Enlow (1963) describes remodeling activities as restricted to surface exposure of bone Thus, the inner surface of a canal containing a blood vessel is subject to remodeling changes in the same manner and by the same means as any periosteal or endosteal surface Many canals are not surrounded by concentric lamellae. Enlow refers to these as primary non Haversian canals or osteons, either radial or longitudinal or conforming to the convoluted pattern of the bone Such primary osteons may be formed directly by newly forming bone Bone which contains clusters of primary osteons is distinctive in appearance This type of osteon is recognized by its concentric circles of lacunae and by the presence of interstitial areas between the osteons characteristically containing non lamellar (woven fibrous, immature) bone Primary osteons may be found in both periosteal and endosteal bone, and will remain as such unless removed by endosteal resorption If they undergo endosteal resorption with formation of a perivascular resorption cavity this cavity then is filled in with new endosteal bone formation forming a secondary osteon or typical Haversian osteon

A process of rebuilding has occurred in the development of the secondary Haversian system, since secondary canals are formed by remodeling of pre existing primary canals which involves the replacement of immature bone with mature lamellar bone

The blood vessels of the labyrinthine capsule are within canals of th primary osteon type In other words, the vessels are surrounded by small areas in which the bone lacunae are arranged concentrically These vessels and their narrow rim of concentrically arranged bone lacunae are separated from each other by interstitial bone which is non lamellar (immature or fibrous or woven bone)

It is this stage of primary osteons and immature woven interstitial bone that presumably remains throughout life in the middle layer of the labyrinthine capsule The primary osteons presumably remain as such with no evidence of remodeling into secondary osteons or secondary Haversian systems. The interstitial bone between these primary osteons remains as immature woven bone

McLean and Rowland (1963) describe the remodeling of osteons as occurring through the formation of resorption cavities or resorption tunnels about the blood vessels. This resorption is typically associated with osteoclasts. The resorption space or tunnel is then filled in with new bone by osteoblasts.

It is true that such remodeling changes are not seen about the vessels of the labyrinthine capsule However certain processes occur about the labyrinthine capsule blood vessels, in all temporal bones at all ages, that resemble to a certain degree the remodeling changes described by McLean and Rowland These perivascular bone changes must be considered to be unique to the bony labyrinth, since as far as the author can discover no other bone in the body has yet been described with this type of "remodeling"

As already mentioned, the blood vessels of the labyrinthine capsule appear to be enclosed in canals of the primary osteon type To a somewhat lesser extent at birth but to a universal degree at all ages beyond, the bone about these blood vessels in all the layers *showed the same changes.* In any one section, the bone immediately surrounding the blood vessels appears to be undergoing changes which vary from one primary osteon to another The bone cells fade and disappear while the bone matrix becomes pale-staining and seems to lose its structure so that the blood vessel is surrounded by amorphous, sometimes granular material containing poorly discernible bizarre-shaped, confluent, empty lacunae Such perivascular areas of matrix depolymerization and loss of osteocytes are present concomitantly with perivascular areas undergoing changes in the opposite direction from the depolymerized appearance to repolymerization of the matrix and with the appearance of new bone cells These new bone cells arise from mesenchymal cells in the perivascular soft tissue which freely enter the depolymerized amorphous matrix Once the mesenchymal cells

enter the altered matrix, they appear to become bone cells, and the area undergoes a repolymerization (and remineralization) back to recognizable bone structure. It appears as if the original mucopolysaccharide matrix is retained in this process, but is alternately depolymerized and repolymerized No actual resorption cavity or tunnel results in this process, and no osteoclasts are seen. If one holds to the usual definitions of remodeling, this process, then is not a true remodeling, since no resorption space or tunnel has been formed, no osteoclasts are seen and the end result is again the same structure a primary osteon rather than a more mature secondary osteon with lamellar bone No replacement of the original structure by more mature structure has occurred. We appear to end up with the same anatomic structure that was present originally Variations in the staining characteristics of the depolymerizing and repolymerizing perivascular bone suggest a greater or lesser amount of depolymerized mucopolysaccharide matrix present at any one time so that bluer areas probably represent an increased rate of "remodeling" about the vessels. A rare temporal bone, uninvolved by otosclerosis, reveals deeply basophilic-staining repolymerizing matrix with new bone cells about groups of blood vessels These vessels (usually in the region of the semicircular canals) all demonstrate the reforming stage of the process, rather than a combination of repolymerizing and depolymerizing phases. These more basophilic, repolymerizing perivascular areas have been referred to as blue mantles. They appear to represent a more rapid reforming of perivascular bone along normal physiological channels. Their presence signifies extensive changes of a degenerative nature elsewhere in the labyrinthine capsule and will be discussed in more detail under a separate heading

The depolymerizing, repolymerizing, waxing and waning process occurs about all the vessels, and all the various stages of this process can be demonstrated at any one time in the same section These changes occur about the blood vessels in all the layers of the bony labyrinth inner endosteal, middle convoluted endosteal, and outer periosteal layers They are not confined to the middle layer alone

A typical picture, as described above can be seen in Fig 6 A The section is from a 19-year old female and reveals the labyrinthine capsule between the cochlea, the canal for the tensor tympani muscle, and the internal carotid artery The bone about the blood vessels takes a pale, eosinophilic stain which causes these areas to stand out sharply from the interstitial bone Perivascular bone matrix is seen in various stages of depolymerization with loss of osteocytes. Note the amorphous or granular appearance of the depolymerized matrix and fading or absence of osteocytes in these areas. A newly repolymerized, perivascular bone area with new bone cells is also seen in this figure Note the deeply staining vessel walls and perivascular soft tissue No resorption spaces or cavities or tunnels are seen, nor are osteoclasts seen Fig 6 B demonstrates similar findings in the otic capsule of a three-year old female Here, too, one sees a typical peri

vascular depolymerizing phase with cell loss and a few new bone cells appearing about the vessel In addition, at this age there are still marrow and loose connective tissue spaces which are being filled in (or compacted) by endosteal cells lined up alongside the bone surfaces Fig 6 C reveals a high power view of a repolymerizing perivascular area in the 3-year old specimen undergoing repopulation with new bone cells One can see the cells arising from about the perivascular soft tissue and entering matrix which is depolymerized, but which contains bizarre confluent lacunae The depolymerized matrix and its contained bizarre lacunae resemble atypical cartilage matrix The new bone formed here is deeply basophilic staining and is similar to *blue mantle bone* The more deeply basophilic nature probably reflects the more rapid "remodeling" occurring during the active growth years. Fig 6 D reveals the bony capsule between the cochlea and the internal carotid artery in a 51 year old female In this particular section the depolymerization phase with loss of cells predominates One can see areas of fading, ghost like" bone cells. Very few new bone cells are evident arising from the perivascular soft tissue However one very small perivascular newly repolymerized and repopulated area is present which stains deeply eosinophilic and stands out sharply within the surrounding interstitial bone Fig. 6 E demonstrates both waxing and waning phases in the labyrinthine capsule of a 55-year old male adjacent to the posterior semicircular canal The depolymerizing matrix here takes a somewhat more blue stain, but otherwise reveals the same type of change as has been described.

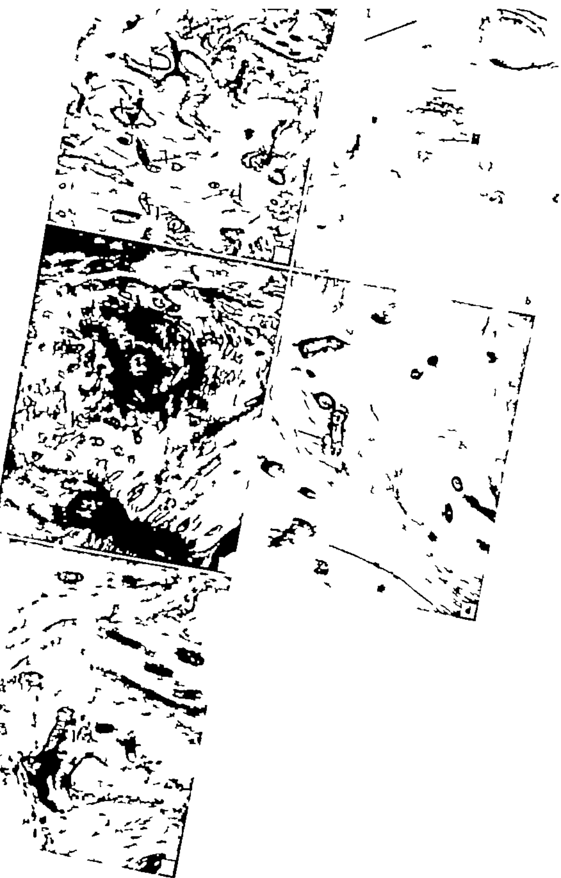
These repolymerizing and depolymerizing, "waxing and waning" changes of the perivascular bone (the primary osteons) bear a certain resemblance to resorption spaces in as far as their location is concerned However they do not result in an erosion space or cavity nor are osteoclasts seen Frost (1963) states that osteoclasts require the presence of mineral in bone matrix to be able to resorb it In this perivascular bone the changes are those of *depolymerization of the mucopolysaccharide matrix which thus breaks the* calcium bonding with release of the mineral, a process referred to as "leaching out of mineral or halisteresis. The newly reformed bone, as mentioned before is again a primary osteon there has been no maturing of the structure in the process.

Von Recklinghausen (1910) believed that the primary change in bone resorption involved the bone cells, resulting in liquefaction (depolymerization) of the surrounding bone matrix. This liquefaction of the bone matrix was then followed by a loss (leaching out or halisteresis) of bone mineral In other words, an in vivo demineralization of the bone matrix. Heller Steinberg (1951) and Bélanger *et al* (1963) demonstrated the relationship of depolymerization of bone matrix to the activity of the osteocytes which occurred in the *absence of osteoclasts. Heller Steinberg* demonstrated that with bone in a more depolymerized state the bone salt becomes more reactive

enter the altered matrix they appear to become bone cells, and the area undergoes a repolymerization (and remineralization) back to recognizable bone structure. It appears as if the original mucopolysaccharide matrix is retained in this process, but is alternately depolymerized and repolymerized No actual resorption cavity or tunnel results in this process, and no osteoclasts are seen If one holds to the usual definitions of remodeling this process, then is not a true remodeling, since no resorption space or tunnel has been formed, no osteoclasts are seen and the end result is again the same structure, a primary osteon, rather than a more mature secondary osteon with lamellar bone No replacement of the original structure by more mature structure has occurred We appear to end up with the same anatomic structure that was present originally Variations in the staining characteristics of the depolymerizing and repolymerizing perivascular bone suggest a greater or lesser amount of depolymerized mucopolysaccharide matrix present at any one time, so that bluer areas probably represent an increased rate of "remodeling" about the vessels. A rare temporal bone, uninvolved by otosclerosis, reveals deeply basophilic-staining repolymerizing matrix with new bone cells about groups of blood vessels. These vessels (usually in the region of the semicircular canals) all demonstrate the reforming stage of the process, rather than a combination of repolymerizing and depolymerizing phases. These more basophilic, repolymerizing perivascular areas have been referred to as blue mantles They appear to represent a more rapid reforming of perivascular bone along normal physiological channels. Their presence signifies extensive changes of a degenerative nature elsewhere in the labyrinthine capsule, and will be discussed in more detail under a separate heading

The depolymerizing, repolymerizing, waxing and waning process occurs about all the vessels, and all the various stages of this process can be demonstrated at any one time in the same section These changes occur about the blood vessels in all the layers of the bony labyrinth, inner endosteal middle convoluted endosteal and outer periosteal layers. They are not confined to the middle layer alone

A typical picture as described above, can be seen in Fig 6 A The section is from a 19-year old female and reveals the labyrinthine capsule between the cochlea, the canal for the tensor tympani muscle, and the internal carotid artery The bone about the blood vessels takes a pale, eosinophilic stain which causes these areas to stand out sharply from the interstitial bone Perivascular bone matrix is seen in various stages of depolymerization with loss of osteocytes. Note the amorphous or granular appearance of the depolymerized matrix and fading or absence of osteocytes in these areas A newly repolymerized, perivascular bone area with new bone cells is also seen in this figure Note the deeply staining vessel walls and perivascular soft tissue No resorption spaces or cavities or tunnels are seen nor are osteoclasts seen Fig 6 B demonstrates similar findings in the otic capsule of a three-year old female Here, too, one sees a typical peri



to be abnormal, leading to the dissolution of bone with bone resorption and the formation of otosclerotic foci.

Weber (1928 1933) recognized the repolymerization and repopulation stage of the bone about the blood vessels in patients with otosclerosis and in patients without typical otosclerosis, and called these areas blue mantles, the name given to them originally by Manasse Weber saw the perivascular blue-mantle changes in all portions of the labyrinthine capsule but saw them more frequently in the region of the semicircular canals, believing them to occur mainly in the middle layer He described the new formed bone as having large, confluent lacunae and resembling primitive bone with cells occasionally resembling cartilage cells (similar to Fig 6 C) Weber believed that prior resorption must have occurred because he could demonstrate by means of polarized light that the fibrils of the surrounding bone ceased exactly where the blue mantles began but he did not recognize the depolymerization process preceding this rebuilding Weber also referred to red mantles and mixed mantles, depending on the staining characteristics, and believed that all three kinds, blue red and mixed mantles, represented new bone formation following previous resorption His descriptions and demonstrations are beautiful The depolymerization process remained un recognized. Weber believed that the blue mantle bone resembled otosclerotic bone, and represented the initial stage of otosclerosis.

Guggenheim (1935) recognized the ability of mesenchymal cells to form bone but he believed that when this occurred in the labyrinthine capsule it was abnormal representing regression to an embryonic state He felt that this "regression whereby the mesenchymal cells formed new bone was genetically determined in susceptible individuals, and the new bone so formed represented otosclerotic bone He did not realize the normal mech anism by which the mesenchymal cells, throughout life, retain the ability to form bone not only in the labyrinthine capsule, but in bones in general

Many authors thus apparently recognized the depolymerizing phase about the vessels (Wolff and Bellucci 1964 Wolff and Lempert 1965 and others) and many recognized or described the rebuilding of bone in these areas (Wittmaack 1919 Nager and Meyer 1931 Weber 1933) but none appeared to recognize both processes, nor was it evident that these were normally occurring processes throughout life, representing an unusual form of "remodeling"

In a previous study (Gussen, In Press) the author described the presence of secondary cartilage (or chondroid) and chondroid membrane bone in the labyrinthine capsule At that time, the normally occurring processes of depolymerization and repolymerization occurring about the blood vessels were not recognized as such, and were described as areas of chondroid cartilage because of the occasional resemblance of the depolymerized matrix to cartilage matrix (Fig 6 C) It was suggested at that time that such peri vascular bone areas represented direct transformation of chondroid cartilage to chondroid bone In a sense the depolymerization of the bone in these

areas reduces it to a more cartilage-like matrix where the mucopolysaccharides are in a lesser polymerized state However it is now recognized that the process represents alternating depolymerization and repolymerization of perivascular bone and that cartilage as such is not present in these areas.

As mentioned before the author has been unable to find any reference to this type of "remodeling" where an actual space or cavity is not produced, but where the matrix reverts to a depolymerized, demineralized state only to be reused in the building back to a repolymerized repopulated area, and where the primary osteon undergoes these changes only to reform as a primary osteon again It would seem logical to assume that a shifting and relocation occurs within the labyrinthine capsule as a result of these processes

BLOOD VESSELS OF THE LABYRINTHINE CAPSULE

The role of blood vessels in osteogenesis has been studied extensively by Trueta (1963) He believed that degenerating chondrocytes, osteocytes and endothelial cells are responsible for the liberation of osteogenic inducing substances, and that such local substances act directly on the vascular system of the bone, stimulating the formation of osteoblasts or their precursors Moss (1960) considers that the osteogenic inducing factor lies in the organic ground substance of bone, that the general group of mucoproteins may be primarily active Autoradiographic studies by Young (1963) and by Tonna and Cronkite (1961) using tritiated thymidine clearly demonstrate the evolution of bone-forming cells from undifferentiated mesenchymal cells.

The appearance of the blood vessels in the labyrinthine capsule is one of continuous activity Their perivascular structure is deeply basophilic, due presumably to increased or concentrated amounts of acid mucopolysaccharide in a state readily available for bone formation. The mucopolysaccharides in these areas, by virtue of their ion exchange ability may hold calcium ions in readiness for the ossification procedure

In almost every specimen above the age of 40 an occasional blood vessel was noted to be occluded by bone deposits. In a few instances, to be described such vessels were seen in somewhat greater numbers

THE INTERSTITIAL BONE

The interstitial bone of the labyrinthine capsule refers to the bone between the primary osteons. This represents all the bone tissue except for the perivascular bone with its concentric lacunae (which make up the primary osteons) and the endosteal and periosteal circumferential lamellae.

The interstitial bone has been shown to consist of membrane bone formed directly by endosteal apposition filling in the marrow spaces. The endochondral globuli ossei are included in the changes to be described in the interstitial bone.

Nager (1947) and Nager and Rüedi (1951) have described the virtual absence of osteocytes in the bone of the middle layer of the labyrinthine capsule. More recent studies by Mendoza and Rius (1966) also describe the widespread loss of osteocytes within the middle layer of the labyrinthine capsule and believe that this necrotic bone is significant in the etio-pathogenesis of otosclerosis.

In studies of bone in general, Sherman and Selakovitch (195) noted that osteocytes begin to disappear from the outer lamellae of Haversian systems during adolescence with the number of empty lacunae increasing after the third decade. Such changes were attributed to circulatory insufficiency and physiologic necrosis of bone with increasing age. Studies by Lindahl and Lindgren (1962) and by Trotter *et al.* (1960) demonstrated that bone density diminishes with age and, that although this is a generalized process, the rate differs in different bones, differs in males and females and differs in the white and Negro races. Enlow (1966) demonstrated osteocyte necrosis in normal bone and found it to be related directly to age and to the extent of the blood supply. Where extensive areas of nonvascular bone were seen corresponding loss of osteocytes was also present. Occasional vascular canals were also seen occluded by bone deposits.

Frost (1963) described loss of bone cells as occurring earliest in the extra-Haversian areas of interstitial bone (which are furthest removed from the vascular supply) and the changes of micropetrosis which may then follow. Micropetrotic bone is dead bone in which bone canaliculi and some lacunae are filled with mineral, and represents the hardest most highly mineralized most radiodense most brittle most impermeable type of bone.

The interstitial bone of the labyrinthine capsule at birth is almost completely cellular (Fig. 1). By three years of age although most of the interstitial bone is still cellular scattered empty bone lacunae are seen throughout all the layers. By 19 years of age, major portions of the interstitial bone

are acellular (Fig 6 A) In these areas, the endochondral globuli ossei and the surrounding endosteal convoluted membrane bone now reveal large rounded empty bone lacunae with smooth walls. Areas of "ghost like" fading osteocytes can be seen scattered throughout Most of the bone cells that are present are in relation to the blood vessels (the primary osteons) which reveal their continuous depolymerizing and repolymerizing "remodeling" processes The periosteal and subperiosteal bone appear to be less involved in the process of osteocyte loss, for in most of the specimens, the outer portions of the labyrinthine capsule appeared to contain mostly cellular bone whereas the inner endosteal circumferential layer revealed increasing loss of osteocytes As the specimens increased in age this loss of osteocytes from approximately the inner half of the labyrinthine capsule resulted in paler staining of this area, so that it appeared to be well demarcated from the outer half of the capsule which contained more viable bone. Fig 6 D demonstrates the almost complete disappearance of bone cells from the interstitial bone in a 51 year old female At all ages, fading ghost like osteocytes are seen The perivascular bone matrix continues to demonstrate alterations in polymerization with bone cells appearing and disappearing but this, too in the majority of the specimens normally appears to become a slower process with increasing age, although still present Certain specimens revealed changes involving the interstitial and perivascular bone which represent an abnormal progression of the changes already described, and these will be presented later

The loss of interstitial bone osteocytes throughout most of the otic capsule layers appears to be physiological since it is present at all ages, without exception Why it should be such a prominent feature of the otic capsule as compared to other bones is not clear It is interesting to note here a study by Crawford (1940) of bone structure in vertebrates In reptiles (lizards and crocodiles) for example, the cells in secondarily deposited endosteal bone were unevenly distributed, with individual lacunae varying considerably in size and shape More significant for us, perhaps, was the irregular course of the bone canaliculi which often did not anastomose with each other so that the bone cells were presumably deprived of nourishment by the tissue fluids, and disappeared The proportion of empty lacunae was unusually high and much greater than in the periosteal bone

Whether non-anastomosing bone canaliculi, or vascular changes with age or both of these (or neither) are the cause of the widespread loss of bone cells in the interstitial bone of the otic capsule cannot be determined at this time

In all of the specimens examined portions of interstitial and inner endosteal circumferential bone were also seen suggesting depolymerization of their matrix with new bone cells appearing (Fig 7 A) This depolymerization of interstitial bone did not normally occur to the same extent as the depolymerization of the perivascular bone New bone cells then appeared to "wander into" the altered interstitial bone from adjacent peri



Fig. 7 (A Depol mer zat on of t ratitial bone with mall numbers f ew bone ell entering the bi rre haped lacunae Not resemblance t cartilage tractar 47 yea old femal H&E 320 R produced from Ame J Anat Gussen, I pres) —(B) Depoly merization f ndorste l reumferent l la er l ing semicircul canal F ding ghost l ke bone l seen w ll as scattered new bone cells, 63-yea ld female H&E 130 Rep duced from Amer J Anat Gu sen, I pres)

vascular bone (primary osteons) or from adjacent soft tissue into the inner labyrinthine layer The amount of interstitial bone "rebuilt" in this fashion varied with age and also varied among specimens of the same age so that in some specimens large areas of devitalized interstitial bone varied with areas of revitalizing or rebuilt interstitial bone. However with increasing age extensive areas of devitalized interstitial bone remained, with only occasional foci of rebuilding or rebuilt interstitial bone evident The periosteal and subperiosteal areas, however usually revealed newly rebuilt bone even in the older specimens

To recapitulate The interstitial bone (endosteal convoluted bone and endochondral globuli ossei) as well as portions of the subperiosteal bone and endosteal inner layer of the labyrinthine capsule, appear to lose their bone cells with increasing age The mucopolysaccharide and mineral matrix of this bone apparently alter to a degree that allows the penetration of new bone cells from adjacent perivascular bone or adjacent soft tissue areas, so that newly rebuilt interstitial bone is present alongside areas of devitalized bone. Many older specimens, however reveal extensive areas of interstitial bone in the inner and middle layers to be completely acellular with little or no evidence of rebuilding

As mentioned before the author in a previous study (Gussen in press) did not recognize the depolymerizing-repolymerizing process as such, and the occasional depolymerized interstitial (Fig 7 A) and endosteal circumferential (Fig 7 B) bone areas which resembled cartilage matrix were thought to represent chondroid cartilage transforming to bone These are now recognized as only resembling cartilage matrix by virtue of their more highly depolymerized state, but actually represent the alternating processes of depolymerization and repolymerization of bone matrix.

Chondroid, or secondary cartilage, transforming directly to chondroid membrane bone, appears to occur only in the surface of the cartilage of the inner layer of the labyrinth As has been shown, the deeper portions of this cartilage (away from the surface) may be replaced by endochondral globuli ossei Once the surface of the inner layer of the labyrinth has formed as chondroid bone this chondroid bone appears to undergo the processes of depolymerization and repolymerization as described

ABNORMAL PROGRESSION OF BONE CHANGES

Two types of change occurred in some specimens which appeared t be an abnormal progression of the normally occurring processes

(1) Extensive depolymerization of interstitial bone As the interstitial bone matrix alters with loss of its osteocytes, the demarcation between the interstitial bone and the perivascular bone in its depolymerizing phase is lost. It appears as if the blood vessel is now surrounded by a large poorly delimited area of depolymerized bone matrix, which is continuous with other perivascular areas having the same appearance Figs. 8 A and 8 B reveal such areas in a 65-year old female and a 6-year old male respectively The blood vessels here no longer appear surrounded by a well delimited cuff of depolymerizing bone matrix, but rather are surrounded by large confluent areas of depolymerized structureless bone These areas of softened, structureless, interstitial bone are occasionally seen as small, incidental foci, usually in older specimens. However as mentioned, large confluent areas of softened structureless interstitial matrix were also seen in the labyrinthine capsule of a 6-year old boy who died of acute lymphoblastic leukemia (Fig. 8 B) Both temporal bones revealed widespread leukemic infiltration of the bone marrow as well as infiltration of the soft tissue structures by leukemic cells. (There was no hearing loss or signs or symptoms referable to the ear during life) The depolymerizing interstitial bone in the 6-year old specimens was present as large irregular confluent areas extending from the periosteal layer through the entire thickness of the labyrinthine capsule, involving all layers, with no evidence of matrix rebuilding. However small numbers of new bone cells were seen scattered sporadically within the pale amorphous matrix, with no evidence of matrix repolymerization either in the interstitial depolymerized areas or in the perivascular areas

It was unusual, however to find such large confluent areas of interstitial matrix softening (depolymerization) without rebuilding (repolymerizing) about some of the bl ood vessels Now however this repolymerization process about the blood vessels is no longer limited to the small area immediately surrounding the vessel but occurs as a concentric repolymerization into a larger area whi h may become confluent with similar concentric rebuilding areas about adjacent blood vessels, so that the interstitial type of bone in these areas seem to have disappeared and seems to have been replaced by coalescing ar as of newly rep lymerizing perivascular bone These newly repolymeriz g repopulating bone areas about the vessels stand out in sharp contr t to the pale virtually structureless bone about them Fig 9

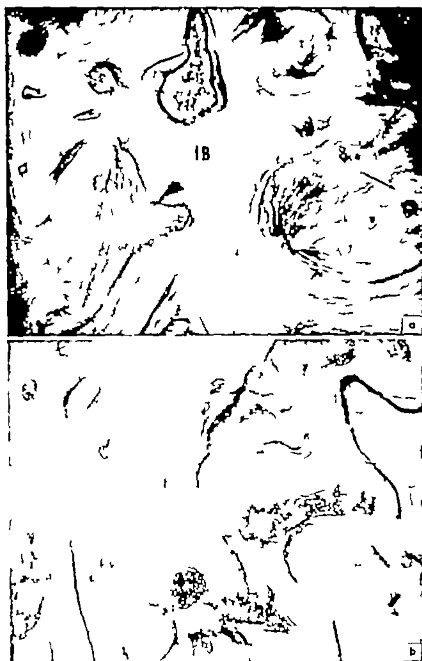


FIG. 8. (A) Labyrinthi ps l dj cent t estibule nea posterior portion f oval wind w N te xtensi depolymerization f interstitial bone with ghost lik f di g bone cells (*IB*) Perivascul bone t right (*arrow*) reveal beginni g repolymerizatio with the ppearance of few new bone cells. 65-yea ld f male H&E 40 —(B) Middl nd subperiost l l rs of labyrinthine capsul in 6-yea ld mal with cut lymph blastic leukemia. Not extensiv diffuse co fl ent reas of ma ked d polymerizati of i ter stitial d peri scula bone with evidence f repolymerizati bo t essels. Gl bull ossei foci are present i right half f photograph. Small n mbers f new bone cell re sc ttered sparsely withi the m rphous d polymerized mat ix. Leuk mic cell re present i the marrow spaces. H&E 40



Fig. 9 Begin ing confl ence f repolymerizing perivascula bone adj ce t t estib l posteromedial t utricl 1 62 ye ld mal Not sharp delineati of repolymerized concentric, und l tl g tward tension f perivascular bo from pal terstitial bo which is irtually structureless l som reas. H&E 40

reveals such an early process posteromedial to the utricle in a 62 year old male. Note the sharp delineation of the repolymerized, repopulated peri vascular bone from the pale interstitial bone The concentric, undulating outward growth is evident, and a few such areas are becoming confluent The newly repolymerized bone itself appears normal the difference apparently being the confluence of such perivascular areas due to the more extensive softening (depolymerization) of the interstitial bone

Fig. 11 A reveals similar rebuilding of perivascular bone in a 79-year old male, adjacent to the vestibule. The newly rebuilt bone is about blood vessels and small amounts of fatty tissue Enlow (1963) in his discussion of the remodeling of endosteal convoluted bone states that when the primary canals undergo remodelling, even though the bone itself is endosteal, the superimposed cylinder of newly forming bone is actually continuous with the fibrous component of the periosteum This may explain the presence of fatty marrow within the labyrinthine capsule which is associated with some of the blood vessels and is surrounded by normally depolymerizing and repolymerizing bone These areas of fatty marrow associated with the



FIG. 10. (A) I terstitial bone changes just beyond globuli ossei f cochlear psul i 79-year ld mal Not diffusely stippled calcification f acell la interstitial bon (micropetrosis) (*arrows*) Tort ou essel f primary osteon (cut twice) is seen in uppe right fl ld occl ded by bon deposit. Perivascula bon i l a d polymerized tate H&E 250—(B) Labyrinthin capsul in angle between cochlea nd i tern l aditory canal in 62 year ld mal Not very m rked, extensive diffuse al ific stippli g f i terstitial and peri ascula bo e reas. Not blood vessel urrou ded by l rg re f calcific d generation with no evidence f repolymerizati (*arrow*) Lam ll e f bone t left line the i ternal auditory canal. H&E 40

primary osteons are not abnormal since they are also found in temporal bones which do not show abnormal changes.

(2) Micropetrosis of interstitial and perivascular bone Many of the adult temporal bone specimens contained occasional foci of acellular interstitial bone which were diffusely infiltrated by deep bluestaining granular or powder like, calcific deposits. However very extensive, diffuse calcification of this type was seen in the specimens of the two male patients, 62 and 79 years of age, who also had shown large areas of softening (depolymerization) of interstitial bone Fig 10 A reveals such a diffusely calcified area adjacent to the basal coil of the cochlea just beyond the globuli ossei in the 79-year old male The adjacent primary osteon in a depolymerized state reveals occlusion of its lumen by bone deposit Fig 10 B demonstrates extensive diffuse deposition of calcific granules in non viable bone adjacent to the internal auditory canal near its culmination at the cochlea in the 62 year old male Here the calcification appears to involve the bone immediately surrounding the blood vessels as well where the perivascular bone and interstitial bone are not distinguishable from each other These

calcific deposits within non viable bone probably represent micropetrosi as described by Frost (1963) where the canaliculi and some lacuna are filled with mineral forming very hard brittle impermeable bon and probably accounts for the hardness so characteristic of the labyrinthin capsule Such evidence of diffuse powder like calcificati n disappears in tissue that has been left too long in acid or has been placed in strong a id for decalcification

To recapitulate Two types of abnormal change are described One in volves a progression of the normally occurring interstitial b ne matrix depolymerization phase so that very extensive areas of vi tuall stru ture less, softened bone are present This may be continuous with th depoly merizing phase of the perivascular bone which also may remain in depolymerized state so that large confluent depolymerized one ar a are evident. In some specimens, repolymerizing bone about the blood essel now rebuilds into larger areas, since the interstitial bone is softened t uch a degree that it offers no barrier to the newly rebuilding bone ab ut the vessels. Such reforming perivascular bone coalesces with ref rming bone about adjacent vessels.

The second type of abnormal change appears to be diffuse calcification of a micropetrotic nature involving the interstitial acellular bone and peri vascular bone areas, forming hard brittle nonpermeable bone

Nager and ... (1931, 1932) described two separate processes that they ... the first stages in the development of otosclerosis, both ... f breakdown and resorption of the old degenerated capsule ... r and Meyer 1931) takes the form of localized resorption ... lacunar erosion followed by "repair" of the area by new ... bone) The second process of breakdown and resorption ... by Nager and Meyer (1932) is by osteoclastic destruction ... ne with the formation of "pathological" bone marrow within the ... paces They believed that such resorption spaces formed ... vessels only in the very beginning of the process, and that afterwar... the resorption very often extended further into the bone without relation to existing blood vessels Rüedi (1963) has pointed out that the cases in the literature where apparent early lesions are described, have had at least one fully established focus of otosclerosis No recognizable early lesions have been described in temporal bones free of definitely diagnosed otosclerosis

In the present study the two male patients who revealed extensive micropetrosis of interstitial and perivascular bone as well as extensive softening or depolymerizing matrix changes, also revealed rare foci representing the two "first stage" otosclerotic processes described by Nager and Meyer These two patients had no history of hearing loss or any manifestation of ear disorders in general Both patients had severe generalized arteriosclerosis the 62 year old patient died of a cerebrovascular accident, and the 79 year old patient who had diabetes mellitus, died of septicemia

Figs. 11 A and 11 B demonstrate the labyrinthine capsule adjacent to the vestibule in the 79-year old male Newly rebuilt bone is evident about the blood vessels and about fatty bone marrow In one such area, the repolymerizing bone only partially surrounds the fatty marrow Part of the fatty focus directly abuts non viable bone which is acellular and contains powder-like calcific stippling A prominent osteoclast is present in this area, its nuclei crowded to one side and its cytoplasm in a bulbous form eroding into the adjacent acellular non viable bone forming a typical Howship s lacuna A similar focus of osteoclastic resorption is seen in a section from the 62 year old patient (Figs 12 A and 12 B) in the labyrinthine capsule between the cochlea and middle ear (near the canal for the tensor tympani) Under low magnification (Fig 12 A) the interstitial bone appears largely acellular and virtually structureless A rare perivascular bone area is undergoing repolymerization with the appearance of new bone cells.

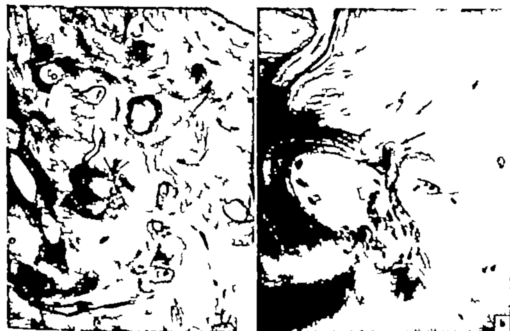


FIG. 11 (A) Labyrinthine peri djace t t the vestib l (pper right corner) in 70-year old mal Not repolymerization f bone abo t blood essel d f tty marrow withi middle layer Not f tty marrow focu ly parti lly urrounded by repolymeriz ing bone matrix with osteoclast (*arrow*) prese t in H wship's lacu a, eroding int acellula calcific interstitial bone H&E 40 —(B) Higher low f f tty marrow focus in middl layer f labyrinthine capsul Not repolymerized bone ly partially surro nd-i g marrow focu with acellular cal ific i terstitial bon directly b tting marrow Osteoclast with n l i bunched i t on rea f cell i seen, with bulbo shaped cyto-plasm of osteoclast in Howship's lacuna, eroding dege erated i terstiti l bo (*arrow*) H&E 150

In one such repolymerizing perivascular bone area (Figs. 12 A and 12 B) the repolymerizing bone appears to surround only a portion of the blood vessel. There is no evidence of fatty marrow Two prominent osteoclasts are plainly seen alongside the acellular interstitial bone. Note the presence of powder like calcific deposits in the interstitial bone. No Howship s lacunae are evident in this focus. No evidence of new abnormal bone forma tion is seen associated with either of the two foci described It is of interest to note here that these specimens from the two patients have in common widespread, diffuse, granular calcification within large areas of acellular interstitial and perivascular bone Small foci of stippled calcific granules were occasionally seen in other older specimens, but they were not present to the extent seen in the two described here It is interesting to speculate here on the presence of such extensive calcific deposit in bone which abuts the vascular areas. As menti ned before physiologic lo s of bone cells with aging occurs first in the interstitial bone, probably by virtue of its being furthest removed from the blood supply With sever blood v el hanges

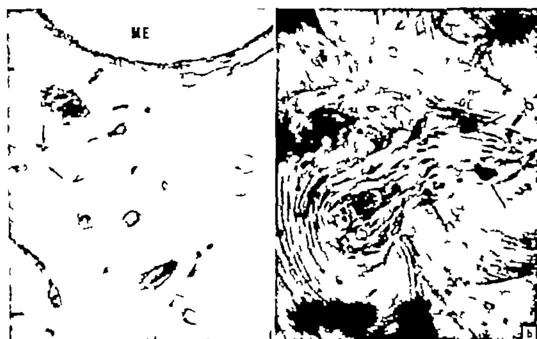


FIG. 12. (A) Labyri thine capsul between cochl d middle ear (ME) (nea canal f tensor tympani muscl) N t acellularity f interstitial bo e with loss f tructure R re perivascular repolymerizati n i evident N t rebuildi g about part f vessel wall with osteoclast present in non-rebuildi g area, adj ce t t micropetrotic bone (arrows) 62-year old mal H&E 40 —(B) Higher iew f primary osteon demonstrat i g repolymerizati n about part f vessel only (on l ft) with tw osteoclasts (arrows) al ngside unrebuildi g porti f bo which co tains diffuse calcifi gra ular mat ial. H&E 250

larger areas of acellular bone result which no longer rebuild and these may now include areas of bone closer to or even about the blood vessels themselves. If perivascular bone, which normally loses its mineral by a process of halisteresis following depolymerization of the mucopolysaccharide matrix,—if this perivascular bone now contains an excess of mineral, or contains mineral filling bone canaliculi and lacunae which are normally free of mineral it is conceivable that the normal depolymerizing, halisteresis process can no longer be effective Frost (1963) has stated that osteoclasts require mineral in bone matrix to be able to resorb it It may be that osteoclasts are now attracted to the area and bring about a breakdown and

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FIG. 13 (A) and (B) Tw foci f lacu erosi n i laby i thi ps le in ngl between i t rn l auditory canal nd basal t rn f cochlea. The blood ess l ppe sur rounded by cell l pale deg nerati g bone with evidence of repolym rizati n The widened, eroded l cuna occur about vessel wall nd extend some distance w y Mesen hym l (bon) cell re present i the wide ed, eroded lac na 62-yea ld mal H&E 125 —(C) H gh power iew f bone N te widened eroded lacu co t i i g mesenchym l cell Sh rply utlined surf ces f the eroded lacun ggest i creased mineralizati f th bo H&E 450.


a


b





FIG. 14 Bo y w ll of horizo tal semicircul ca l Not irregul erosi f bo rface with presence f mesenchym l cells withi the d fects. Thin mucosal ll l g f periotic space tretches cross the origi l u eroded lt 62- ea ld male. H&E 280

resorption of the bone which can no longer undergo its normal depolymerizing process.

The demonstrated foci appear to be examples of Nager and Meyer's osteoclastic type of resorption representing the early lesion. They occur here in temporal bones which have demonstrated widespread degenerative changes within the interstitial and perivascular bone both of a calcific (micropetrotic) nature and of a depolymerized nature where evidence of minimal confluence of normally reforming perivascular bone is also present *It is remarkable to note that in both of these patients, the stapediovestibular* joint was normal, showing only minimal changes of aging (to be presented as a separate study)

Two separate foci of lacunar erosion as described by Nager and Meyer were found in the 62 year old specimen (Figs 15 A B and C) These lesions were present in the angle between the internal auditory canal and the basal turn of the cochlea The blood vessels here are close together and appear to be surrounded only by non viable interstitial bone, with no evidence of concentric perivascular bone Portions of this acellular non viable bone about the vessels appear to be breaking down by means of lacunar erosion with widening and confluence of the lacunae The lacunar erosion is present about the vessels, and extends for a short distance away from the vessel walls. Mesenchymal cells from about the blood vessels have entered some of these erode l c nfluent lacunar areas. The sharply outlined surfaces of the eroded la n ie ugge st that they were more highly mineralized than



FIG. 15. Portion f terior margi of rou d window demonstrati g deep erosion (rrow) Not lin of calcific material stretching across original neroded m rgin. The rface bo lining is deeper-stai i g. d ppears more newly formed (or remodeled) than adjacent caps le bone Not mesenchymal cell activity t margins of round window 62-year ld mal H&E 450

other portions of the acellular bone One can speculate here too, on the possible role that such increased abnormal mineralization may play in the breakdown of bone which normally does not break down and resorb, but depolymerizes and loses its mineral by a process of halisteresis.

These foci strongly suggest the process of lacunar erosion and beginning bone repair by perivascular mesenchymal cells described by Nager and Meyer Whether such lesions as these would have progressed eventually to recognizable full blown otosclero is, or whether they would have "repaired" as small, limited foci of gradually maturing new bone without the development of otosclerosis, is not known

In addition to th foci of osteoclastic resorption and lacunar erosion, a third type of rosion was seen in the 62 year old male patient. Irregular erosions of the inner circumferential endosteal layer in the region of the vestibule and h rizontal semicircular canal were quite prominent (Fig 14) Small number of mesenchymal cells were present within the erosion de fects. The thin mucosal lining of the periotic pace can be seen stretching across the or nal uneroded lte Such occa i n l mall eroded areas within th inner lining of the labyrinthine capsule w r found in a good number of adult spec men One mu t consider th p bility of this type of erosion having a s milar significance to the pre i ly described lacunar erosi n and that "repair" of these area might pc l ly result in a limited new bone

deposition within mesenchymal tissue or might possibly result in a widespread new bone growth representing an otosclerotic focus One must also speculate here on the possible role that the constantly moving inner ear fluids may play in the production of such surface erosions

The round window margins are also formed by membrane bone In the same 62 year old male patient, extensive erosion of the anterior margin of the round window was also present (Fig 15) Note the deep erosion with a persisting remnant of calcific material stretching across the original site of the round window margin The bone lining this area appears to be more newly formed (or remodeled) and stands out in sharp contrast to the adjacent capsular bone Mesenchymal cell activity at the round window margin is evident

Occasional small erosions of the round window were not uncommon in the adult specimens, and were usually associated with repair by mesenchymal cells It was also very common to find acellular bone spicules, with an in-between appearance of cartilage-bone matrix, extending from the anterior margin of the round window into the adjacent cochlear spiral ligament with occasional spicules forming a thin, shell like surface on the spiral ligament

OTOSCLEROSIS

Two specimens were from a 49-year old white female with typical bilateral otosclerotic ankylosis of the stapedial footplate (A study of the normal stapediovestibular joint and the abnormal progression of certain changes to otosclerosis will be presented as a separate study) The same mechanisms are believed to be active in otosclerosis involving the stapediovestibular joint including the fissula ante fenestram save for the very significant fact that the stapediovestibular joint by virtue of being a joint, is subject to the stresses that all joints are subject to, which predispose this area to otosclerosis. The otosclerotic specimens are only partially discussed here for comparison with certain features already described Fig 16 A demonstrates ankylosis of the anterior portion of the stapediovestibular joint The section is through the edge of the ankylosed area to demonstrate the annular ligament The otosclerotic bone involves the anterior portion of the stapes footplate and oval window and extends to both the middle ear and vestibule surfaces, as well as "invading" the otic capsule toward the cochlea Fig 16 B demonstrates the typical appearance of "active" otosclerotic bone Note the large deeply staining mesenchymal cells in the annular ligament adjacent to the new bone Similar cells are present in the closely spaced bone lacunae. The new bone is membrane bone which has formed in mesenchymal tissue Fig 16 C is a high power view of the advancing front of the otosclerotic focus into the labyrinthine capsule as seen in Fig 16 A Note the sharp line of demarcation between the new bone and the old capsular bone, and the undulating type of growth into the older bone A comparison of this "invading" type of bone (Fig. 16 C) with the otosclerotic bone of the oval window (Fig 16 B) reveals a marked difference in appearance The new bone involving the joint is more densely cellular and the mesenchymal cells are forming new bone within mesenchymal or fibrous tissue whereas, in the advancing front of new bone (Fig 16 C) the mesenchymal bone-forming cells appear to enter altered matrix of the adjacent old capsule with repolymerization of the matrix. There is no formation of bone here within fibrous tissue as in the otosclerotic bone of the joint (Fig 16 B) The invasive bone demonstrated in Fig 16 C appears to spread in a manner similar to the normally occurring but more limited process of depolymerization of bone matrix with entrance of mesenchymal cells into the altered matrix which then, as bone cells, bring about a repolymerization and remineralization of the area Here however in the otosclerotic specimen, the bone change occurs about the vessels as an invasive

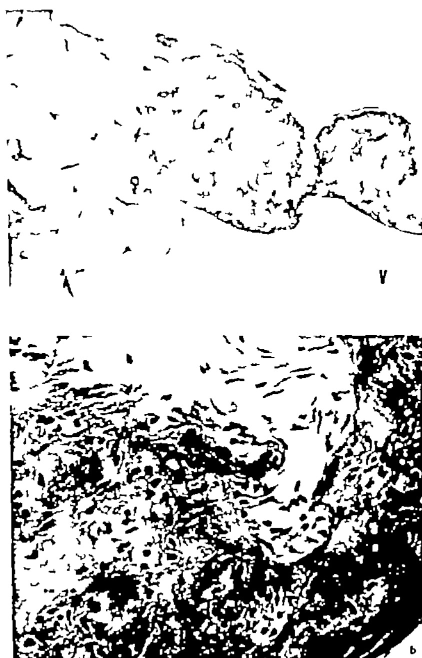


FIG. 16 (A) St pediovestibul j l t, teri with tosclerotic kylosis. Secti n l from edg f nk losed porti t demonstrat on la ligame t. V = Vestibul 49-year ld femal H&E 25 —(B) Highe iew showi g typical active otosclerotic bone N te large, deeply st ini g, mesenchym l bone-formi g cells i the la ligament djacent t new bo Similar cell are present i the losely paced bo lacuna New bone ha formed i mesenchym l ti H&E 4.0 —(C) Spreading front f otosclerotic focus int



labyrinthine ps 1 Note sharp line f demarcati between newly formed bone and old caps la bone Not rra gement f bone about blood essel and resembl nce to coal seing as f repolymerizing peri seular bone H&E 280 —(D) [illegible] otosclerot pecimen) adjacent t semicircula canal Not the [illegible] seular m t l which is beginning t be repolymeri ed nd [illegible] Not occ sion l ew bon cell in interstiti l bone H&E 125

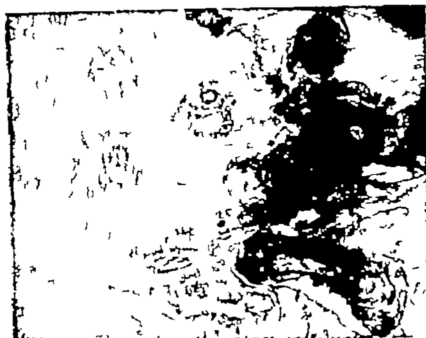


FIG. 17 Bl m s l n -otosclerotic specimen xhibiting extensi micropetrosis d osteocl tic focus lsewhere New bon cells are ntering basophilic t ini g de-polymeri ed per ascul bon Some new c ll have entered i terstitial bone 79-year ld m le H&E 125

front with extensive matrix change preceding it and with confluence of the perivascular rebuilt bone so that the interstitial type bone is no longer present as such

One must recall here that Wittmaack (1933) and others believed that part of the otosclerotic bone consisted of degenerated old capsular bone The invasive front of the focus into the capsule, as demonstrated here is consistent with this view However in the area where the otosclerotic process originates by breakdown and resorption of bone, the otosclerotic bone forms within the fibrous or mesenchymal tissue filling the resorbed or eroded areas, and not in depolymerized matrix, and does not utilize the old capsule since the old capsular bone in such areas has been resorbed or completely broken down New definitions may be necessary to distinguish the otosclerotic bone forming in eroded fibrous containing areas from the repolymerizing, coalescing perivascular invasive type of bone which utilizes the more normal processes of the area

What has been referred to in the literature as blue mantle bone appears to represent a rapid rebuilding phase of the softened altered matrix about blood vessels. Variations in the staining characteristics that occur in the depolymerizing and repolymerizing perivascular bone suggest a greater or lesser amount of depolymerized mucopolysaccharide matrix present at any one time so that bluer areas probably repr ent an increased rate of "re-modeling about the vessels. In otoscleroti pecimens, this may well reflect

a rapid attempt to rebuild along normal channels in areas away from the focus to compensate for the breakdown in adjacent pathologic area or areas. Fig 10 D demonstrates blue mantles in one of the specimens with otosclerosis. When these blue mantle areas are seen in specimens without established otosclerosis, they probably represent an attempt at rebuilding bone to compensate for extensive bone changes of a degenerative nature in adjoining areas of the capsule Such blue mantles were present in sections from the 79-year old male with demonstrated extensive micropetrosis and beginning osteoclastic resorption (Fig 17) Blue mantle bone per se is thus probably not abnormal bone since it follows the same principles of rebuilding within previously softened matrix about the vessels However its presence about a group of vessels in any one area signifies extensive degeneration of bone elsewhere and probably represents an attempt at more rapid rebuilding of normal bone as a compensatory measure

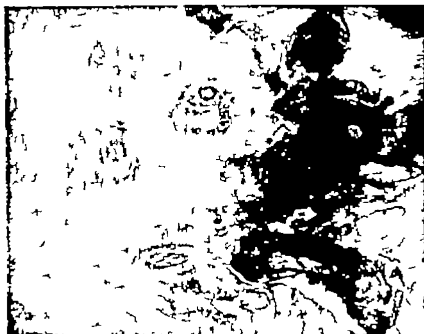


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S U P P L E M E N T U M 236

CELLULAR PATTERN AND NERVE SUPPLY OF THE HUMAN ORGAN OF CORTI

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From the Ear Nose and Throat Department University of Göteborg Sweden.
(Head Professor Gösta Herberts M D)

ACTA OTO LARYNGOLOGICA

SUPPLEMENTUM 236

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GÖTEBORG [illegible]

R TADIL BOKTRYCKERI AKTIEBOLAG
GÖTEBORG 1964
[illegible]

CONTENTS

To my wife, Gunilla

Symbols used in the graphs of sensory cell population

 Full complement f oute hai cells (range i 7 fetuses)

 Outer hair cell

 Full density f each f the 1st, 2nd and 3rd rows of outer hai cells

 1st row of outer h i cells

 2nd " "

 3rd " " "

 4th

 Full complement of inner h cells (ra ge in fetuses)

 Inne hair cell

Symbols used in the audiograms

 Right i conduction

 with masking

 bone conduction

 with ma ki g

 Left ea i conduction

 bon conductio

I. INTRODUCTION

Recent decades have seen rapid ad ances in the both methodology and interpretation. Instrume auditory testing have been developed to a degree assessment of many parameters of hearing. A he f rms of hearing loss as expressed in t rm of tinguish with considerable confidence between c of auditory defects T rms such as recruitment commo ly used in discussions of hearing loss eating damage to sensory cell f th organ of ask the audiologist o the tologist to describe organ of Corti, in any particular case of hearing if any could gi e even a reasonably approxima

The present-day urbanized environment is wh ch const tute an ever-present threat to hearing of noise producing machines and a number of gical features of hearing loss due to noise expo is a cons derable amount of inf rmation on noise-exposed experimental animals though man there is som kn wledge though much on ha cells and neural elements however from satisfact ry

In 196_ Rosen et al reported a study of tribe in the Sud n which had never been expo ty noise found in the ci ilized w rld The frequencies was much better among the old (of a comparable age group) It remains to t e lack of high frequency hearing loss is dep free surround ng. Thi quest n can only b f ll the other ariables between the two ceetera) Nevertheless, the evidence produ to the hypothesis that presbycu is can be ex at least n some degree fr m hronic expo cochlear damage of epidemic proportions populations Regarding t e morphological ports h e demon trat d certain histologic f the cochlea a attributed t presbycu ne precise nf rmation about sensory ments in the organ f Corti

The rapid developm nt f new antibio

while clinically valuable have the unfortunate side effect of causing serious damage to the auditory or vestibular endorgans, thus limiting their clinical use Many reports appear in the literature on the vestibular and/or auditory disturbances attributable to the ototoxic antibiotics. Experimental research on this problem has yielded a considerable fund of facts, but it is only very recently that information on the exact location and pattern of distribution of the damage to the sensory and neural elements has begun to accumulate Drug ototoxicity to the auditory mechanism in man has been studied mainly as an audiological follow up of antibiotic therapy and corresponding histological studies yielding important information on the actual cochlear damage are very few

The human fetus, especially during the first three to four months, is very sensitive to a variety of noxious agents, exposure to which may result in congenital malformations For example rubella infection of the mother within the first three months of pregnancy may induce malformations of the inner ear Congenital malformations of the inner ear also may be the result from inherited factors as for instance in congenital hereditary deafness, nonendemic goiter Usher syndrome and Alford syndrome Whilst there is some useful information about the gross changes in the bony labyrinth in cases in which osseous changes have occurred there is a conspicious lack of detailed inf r mation about the changes occurring in the sensory cells of the organ of Corti and its related neural structures in cases of congenital malformation

Our knowledge of the structural basis of hearing loss in man is slight compared to the enormous amount of research effort which has gone into this problem This is not to say that previous studies have been without value They have in fact, given us basic information on a number of facets of the problem; however there are very few data on one central issue, which is to determine the pattern of damage to the cochlear sensory cells or to the nerve endings and nerve fibers There is a corresponding lack of specific information as to the correlation of sensory cell losses and neural losses in cases of hearing defects which are considered due to morphological changes in the cochlea If we accept the fact that there is a need to improve our knowledge about the adult cochlea this is even more true for the fetal cochlea

There is a good reason to believe that many of these gaps in our knowledge are attributable partly to the methods which have been used and partly to overall difficulties in the preparation and study of the inner ear More recent developments in ultrastructural research have clearly shown up the deficiencies of some of the traditional techniques whilst new techniques of fixation embedding and structural analysis have demonstrated the extreme importance of taking into account all possible artefact Some of these problem have been discussed in a recent monograph by Engström Ades and Andersson (1966)

The preparati n of th human cochl a is beset not only by all those diffi culties found n expe mental anim ls, but also by even greater problem peculiar to th hum n It i these which have presented the greatest obstacle

to this study It is hoped that the soluti ns to some of these technical problems w ll be found useful to the reader and will lead to improvement in our knowledge both of the n rmal and of the pathologically altered human ear

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II SURVEY OF LITERATURE CONCERNING THE HUMAN ORGAN OF CORTI

A INTRODUCTION

The discovery of the cochlea was made by Gabriel Fallopius in 1561 and the acoustic papilla was described for the first time by Alphonso Corti in 1851 These basic descriptions were followed in the second half of the 19th century by many thorough investigations of the anatomy of the cochlea by Kolliker (1852) Leydig (1857) Deiters (1860) Hensen (1863) Retzius (1884) and others The early literature is thoroughly reviewed in the monumental work by Retzius in 1884 In the 20th century Held (1926) and Kolmer (192) have contributed exhaustive descriptions of the morphology of the human cochlea

The technique of decalcification, embedding, and sectioning of the temporal bone was a relatively late development, not known to the early workers Instead they worked with dissections of the ear which were prepared in various ways for direct observations or light microscopy The surfaces inside the labyrinth were especially suitable for this kind of study Several accurate descriptions of the pattern of supporting and sensory cells of the organ of Corti illustrated by excellent drawings, appeared among the early efforts, especially those of Retziu (1884) and Held (1926) Retzius used a technique of preparation which was similar in many ways to the one used in the present publication He preserved the natural pattern of sensory cells and supporting cells and enhanced its clarity by the use of fixatives and stains, so that he could study in detail the surface mosaic of the epithelium These method were supplemented by a sectioning technique

The advent of more sophisticated sectioning methods including that of serial sectioning gave a considerable impetus to the study of cochlear morphology as also to other histological studies as well However the gains of these methods were partially offset by a certain loss, namely a sacrifice of the survey effect of the whole-mount method which helped to maintain one's orientation and perspective This disadvantage was only partly compensated by such devices as sectioning in different planes and the use of alternating thin and thick sections A further great addition to the usefulness of sectioning methods was made in Guild's description (in 1921) of a method of graphic reconstruction from serial sections for the study of the guinea pig's organ of Corti It was the first effort to standardize methodology for the study of cochlear structure and pathology The method was further developed and adapted for the human cochlea by Guild *et al* (1931) Crowe Guild and Polvogt (1934) and in recent times by Schuknecht and his collaborators (19 3a 196 196 1968) It is now a standard method in many temporal bone laboratories

During the la t 50 e rs, the method of serial sect on ng of temporal bones has been th method used almost exclus vely n the tudy of the morphology and patholog f the inner ear Devoti n to this technique w th th disadvantages nherent n an single method, has tended to retard progress in the area of cochlear histopathology The development of electron microscopy has result d *in enorm us ad ances and has evealed many new aspects of struc*ture and funct n whi h have f rced us to revise many of our old concepti ns

The application of electr n m croscopy to the study f the inner ea soon reve led that pec mens which had been decalcified and embedded accord ng to the trad tional techn que could not be used with goods results conse*qu ntly a technique of preparation had to be dev loped in which th* inner ear could be fixed by direct perfusion with a su table f xati any selected part of the inne ea epith lium processed without decalcification, and the specimen then embedded and sectioned (Engstr m and Wersall, 19 3 b) *This led to the reviv al n part of ld micro-dissect n methods wh ch led n* turn to the realization that these methods could be useful for other kinds of tudies in add tion to electron m croscopy Sev ral investigat l ave used s m l r techniques of micro-dissection Neubert (19 0 19 .) described a method f H utchen-preparate in which, after lay ng free th organ of Cort the *nuclei of the sensory cells could be tud ed by l ght microscopy* Got Ito and Ohashi (1953) and Katsuki and Co ell (19.3) used mila methods as did Vinnikov and Tit va (1963 1961) in th ir two-dimens onal preparations f histochem cal stud es Engström *et al* (196 1964 1966) dev loped the *surface specimen technique f r phase contrast microscopy of the organ of* Cort In th study of the human cochlea Kimura Schuknecht and Sando (1964) introduced lectron mi roscopy Bredberg Engstr m and Ades (1965) Bredberg (196) and Johnsson and Hawkins (196) applied the surface specim n technique nd Nomura nd Schuknecht (1965) Nomura nd Kirika (196) nd Ishii Murakam and B logh (196") used hi tochemical methods

B FETAL DEVELOPMENT

The membran us lab rinth arises as an invagination of the ectoderm The e rl est prim rdi are thickenings f the ectoderm, appearing one on either de f th brain. These audit ry placodes becom inv ginated t f rm audi t ry sacs whi h are later separated from the surface to f rm the uditory *vesicles otocysts n the 4—6 mm human mbryo*

Anson (1934) mpha ized three infold ngs of the wall f th otocy t which ubd ide t into the prin pal subd isions of the final membran us laby rinth, n m ly 1) th end lymph tic duct nd s) the utricl and semi*rcula ducts, and* 3) th saccule with its utgrowth, the ductus reuniens and the cochlear duct

Th dev l pment of th sensory epithel um of the cochle r duct i de scribed t geth r w th a evi w of the pert nent lit rature in the f ll wing paragraphs F further *tudies on th* dev l pment of the lab rinth the

reader is referred to the monograph by Bast and Anson (1949) where the earlier literature is reviewed

The cochlear duct is a helical tube which develops as an outgrowth from the antero-medial end of the saccular portion of the otocyst In early fetuses, up to the age of eight weeks it is the only part of the cochlea present The scala vestibuli the scala tympani the modiolus and the bony capsule develop later from the surrounding mesenchyme

The cochlear duct in the seven-week fetus has developed into a curved tube which describes one turn At the 30 mm stage (8 1/2 weeks) it has increased to one and three-quarter turns. At the 50 mm stage (10 to 11 weeks) the definitive two and three-quarter turns are present Beyond this stage the spiral grows in size, but not in tortuosity reaching its maximum size at about mid term (Bast and Anson 1949)

Differentiation of the epithelium which forms the organ of Corti is a process which does not proceed uniformly throughout the coils of the cochlea Most authors have stated that the development begins at the basal end and proceeds gradually towards the apex (Retzius, 1884; Van der Stricht 1919 19?0; Wada 19_3; Alexander 1926 Held 19?6; Kolmer 1927 Bast and Anson 1949 Weibel 1957) Lorente de No (1933 a) described a somewhat different pattern in the mouse in which the differentiation began at a point some little distance from the base and progressed in both directions from there In agreement with this, Larsell McCrady and Larsell (1944) found that in the opossum the morphological differentiation in the upper basal and lower middle coils preceded that in the basal and apical extremities. The functional significance of this was suggested by the finding that the pouch young opossum reacted earliest to sound stimuli in a relatively narrow frequency band in the middle range only later beginning to react to lower and higher frequencies In the rabbit Anggård (1965) showed by electrophysilogical methods that the earliest sign of cochlear function occurred in response to stimulation with medium high frequencies (_—5 kc) Similar observations have been made in the rat (Crowley and Hepp-Reymond 1966) and in the mouse (Mikaelian and Ruben 1965)

A different a pect on cochlear development in the mouse was advanced by Ruben (196?) in a study of terminal mitoses of the cells in the membranou labyrinth by analysis of radioautographs of the inner ear following labeling with tritiated thymidine Terminal mitoses are considered to be the last divi sion which a cell undergoes and thus serve a an index of the time of establ hm nt of a permanent cell population Ruben found in the cochlea that th hair cell (and pillar cells and Deiters cell) were distributed in such a way that the oldest cell the cells that undergo terminal mitoses first, were at the apex and the youngest cell the cells which undergo terminal mitosi la t were at the l se This would infer that the api al cells take longer time after underg ng t rminal mito to reach their mature form than do the hair cells loc ted in the l asal turn Ruben suggested that the growth area in the organ of Cort might be t the l a al end so that growth by cell division would

cause the ap cal part, whose cell-divis on is completed, to be pushed further apicalward

In the human fetus the development is described as starting at the basal end of the cochlea (Alexander 19 6 Kolmer 19‾‾; Bast and Anson, 1949 Ormerod, 1960) In the beginning of th third m nth the cochlear duct has, in cross sect on, an oval shape which later becomes triangular when Reissner membrane begins t separat from the surrounding mesenchyme The first sign of development of the organ of Corti is a thickening of the epithel um n the l we part of the cochlear duct. This is later divided into two ridges, a larger inner ridge and a smaller outer one. The acoustic papilla is d fferentiated from the tissues at the junction of the ridges Light and dark cells can be distinguished at an early stage The dark cells differentiate to become hair cells, th light ones to become supporting cells. Kolmer (19_) expressed the opinion that by this st ge no further m t tic divis ons occur and that therefore, subsequent growth must occur by cell growth and furthe differentiation Th inner hair cells show earlier differentiation than the outer especially as seen by sections through the surface of the organ of Cort (Kolmer 1927) which rev als the earlier appearance of the sensory hairs The acoustic papilla grows rap dly in size as the intra-ep thelial fluid paces are formed and widened The formation of the fluid spaces has been studied in animals especially by Van d r Stricht (1919) and W ibel (195) The initially narr w intercellular clefts expand to become relatively w de intraep thelial spaces, whil the supporting cells change in shape to become m re slender Van der Stricht (1919) considered the flu d spaces to be formed by reducti n of the volume of the supporting cells through cyt lysis and l que faction The subsequent widening of Nuel s pace cannot be explained by diminishing cell bodies, but is due instead to real growth of the supporting cells nd concomitant expansi n of the pace by the secretion of fluid (V n der Stricht 1919; We bel, 195) At this time the cytoplasm in the nner and outer pilla cells is richly vacuolated

The tunnel of Corti appears later than th space of Nuel In th human fetus it has become discernible in the basal coil at 1o weeks of age (Bast and Anson 1949) During the succeeding m nth the development is gradually ex tending to the apex At the f tal age of 5 weeks the cochlea has attained ts final ize and is fully nclosed in the bony capsul At this tage the o gan of Corti resembles that of th adult (Bast and Anson, 1949; Ormerod 1960)

The limbus piralis and the inner p ral sulcus are f rmed from the inn ridge The outer piral sulcus is formed fr m the outer part of the outer ridge The tectorial m mbrane appe rs a a j lly lik depos t on the inner ridge Later it is separated from the cell surface between the limbus and the nner hai cells. Acc rding to Weibel (195) the fluid under the tectorial membran is f rmed by the inner sulcus cells which are richly vacuolated at this t me

Kikuchi and Hilding (1965) have studied the dev lopm nt of the o gan f Corti in the mouse by elect on microscopy They found that inner and

outer hair cells Deiters cells pillar cells nerve fibres, and afferent nerve endings can be identified at birth Both sensory and supporting cells are provided with a kinocilium The stereocilia are formed on the sensory cells and microvilli are numerous on the supporting cell As development proceeds, the kinocilium of each hair cell disappears though the basal body remain as a remnant The microvilli diminish in size and number The development of the tunnel of Corti and the other fluid spaces is completed on the 10th day On the same day the efferent nerve endings appear and by the 14th day the organ of Corti appears well developed as seen by either light or electron microscopy

In a 24 week human fetu Wersall and Flock (196) found in electron microscopy a cilium to protrude from the centriole of the outer hair cells

Bredberg, Engström and Ades (1965) and Bredberg (1967) have studied the development of the surface pattern of the organ of Corti in the human fetus A full description of this development will be given in chapter V in the present publication The development of the surface pattern of the organ of Corti in other mammals was described by a few authors at the beginning of the 20th century (N Van der Stricht 1908 guinea pig and bat; Held 1909 rabbit O Van der Stricht 1918 19 0 rat and pig) In these animals the development is reported to begin at the basal end of the cochlea The first sign of differentiation is a modification of the homogenous pattern of epithelial cell in the area of the inner and outer ridges and the appearance of inner hair cells in the inner ridge Somewhat later the inner pillars can be identified and still later the upper surface of the outer hair cells appear as small polygon l fields which take a darker stain than the supporting cells At the apical end of the cochlea this development takes place at a later time A liplosome i apparent on both supporting nd sensory cells. These studies were based on sections in the plane of the surface of the organ of Corti and are illustrated with a number of excellent figures of the surface pattern in the org n of Corti

The development of the innervati n of the organ of Corti ha been most thoroughly studied by Retzius (1891) Cajal (1919) Lorente de No (19 6) and Tello (1931) At a very early age even before the two epithelial ridges appear a subepitheli l plexus of nerves can be discerned Lorente de No lemonstrated in the rat fetus that the nerves have estal lished connecti n with the sensory cell by the time these can be first identified in a section (11 mm rat fetus, basal coil) He expressed the opinion that the nerve fibres nd the sen ry cell levelop indepen lently up to a certain point but are th reafte int rdependent for the completion of their devel pment Nerves and sensory cell establi h contact with each other at about th same tim a th perilymph t p ces beg n to be formed (Lorente de N 19 6)

The myel nation of the n rves occurs much later Bechterew (188) f und that the m l n t n of the cochlear nerve in the human fetus t kes place at a fet l length of 30 m Lorent d N (19-6) indicated that in the rat m t of the nerv ju re il l n he th on the fifth lay aft r birth

It is important in the study of development and differentiation in the organ of Corti to know when the ear starts to function as a hearing organ. In the opossum Larsell, McCrady and Larsell (1944) correlated the beginning of auditory function with the development of the tunnel of Corti. Kikuchi and Hilding (1965) concluded after comparison of their own observations in electron microscopy with the electrophysiological studies by Alford and Ruben (1963) that in the mouse all major structures are well developed before auditory responses can be elicited. The development of the efferent innervation was found to be the last major event before the organ of Corti begins to function. They suggested that the efferent innervation may play an important role in the function of the maturing organ of Corti.

A few observations of auditory function in human fetuses have been reported. Fleischer (1955) noted movements of the — to 9 months old fetus following a tonal stimulus delivered some distance from the abdomen of the pregnant woman. The stimulus intensity was 115 dB and the frequencies which induced reactions were 500 cps and 1000 cps. Murphy and Smyth (1962) and Johansson, Wedenberg and Westin (1964) noted with the aid of ECG a rise in the fetal heart rate following tonal stimulation. In fetuses at a time — weeks before term Johansson, Wedenberg and Westin (1964) observed this reaction in response to a sound stimulus (3000 cps 110 dB) delivered on the abdomen of the pregnant woman. Wedenberg (1965) reported that such reaction had been possible to record in the 26th week of pregnancy.

C. THE MATURE ORGAN OF CORTI

The inner ear is enclosed in the petrous portion of the temporal bone. It forms a system of canals and cavities surrounded by bone. The bony labyrinth encloses the membranous labyrinth, a system of tubes and vesicles filled with a fluid, the endolymph. The perilymphatic space between the bony and membranous labyrinth is filled with perilymph, a fluid differing in chemical composition from the endolymph.

The bony labyrinth consists of a central part, the vestibule, which encloses the utricule and saccule. From the vestibule rise the three semicircular canals with their dilatation, the ampulla. The anterior part of the vestibule continues into the cochlea. The vestibular part of the membranous labyrinth includes five areas of sensory epithelium: the macula utriculi, the macula sacculi and the three cristae ampullares. Within the cochlear part the sensory epithelium runs along the cochlear duct.

The cochlea consists of a spirally curved tube, the cochlear canal, which forms two and a half to two and three-quarter turns around its axis with the radius decreasing towards the apex. The cochlea measures about 5 mm from base to apex with the greatest diameter of about 9 mm at the base. The apex is directed forward, laterally and slightly downward. The cochlea is located

medially to the middle ear and the promontorial wall forms the partition over the lower basal coil

In descriptions of the cochlea certain conventions have come into use for describing the directions and position of one structure in relation to another These terms are referred not to the body as a whole but to the cochlea itself Thus the term upwards refers to an ascending direction in the plane of the cochlear axis. A direction following the course of the cochlear canal is called spiral and a radial direction from the axis of the cochlea is called outwards

The cochlear canal courses spirally around the modiolus, a short and broad conical pillar of spongy bone The base of the modiolus forms the bottom (i e distal end) of the internal acoustic meatus where the cochlear division of the VIIIth cranial nerve penetrates into the cochlea through numerous openings in the bone Within the modiolus the nerve bundles spread out t Rosenthal s canal where the spiral ganglion is situated

The bony cochlear canal is divided into three spirally running spaces an upper space the scala vestibuli; a middle space which is called the cochlear duct or scala media and a lower space, or scala tympani The scala vestibuli and scala tympani are perilymphatic spaces which communicate with each other through the helicotrema a small opening at the apex At the basal end of the cochlear canal the scala vestibuli opens into the vestibule close to the oval window which i separated from the middle ear by the innermost ossicle the stapes The scala tympani ends blindly at the round window which is separated from the middle ear cavity by a thin resilient membrane (the secondary tympanic membrane) Just inside this round window membrane a bony canal, the cochlear aqueduct opens into the scala tympani Through this canal the perilymphatic duct extends to the subarachnoid spaces

The cochlear duct is part of the membranous labyrinth Close to its basal end the caecum vestibulare it communicates with the other endolymphatic spaces through a narrow duct, the ductus reuniens At its apex the cochlear duct has a blind end the caecum cupulare

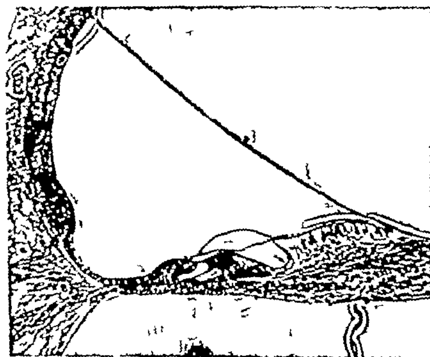
A seen in a section in the mid modiolar plane the cochlear duct is triangular in shape (Fig. 1) It i separated from the scala tympani by a spirally running partition the inner part of which consists of a bony plate, the osseous spiral lamina projecting from the modiolus The outer part of the partition is formed by the basilar membrane a filamentous membrane suspended between the osseous spiral lamina and the spiral ligament The latter covers th outer wall of the cochlear canal and form a rather prominent layer of connective tissue toward the base The basilar membrane is narrow at the base and becomes broader toward the apex. In the most basal few millimeters of the cochl the area of attachment of the basilar membrane to the spiral ligament upported by m ll bony l mina the secondary osseous spiral lamina wh projects nward from th outer wall of the cochlear canal

Th d t separated from th scala estibuli by Reissner s mem-

Ict ed

brane formed by two thin layers f cells one ectodermal and one mesodermal, and separated from each other by a basement membrane

The out r wall of the cochlear duct is formed by a thi k epithelium, the stria vascularis covering th spiral ligament. It is a complex ep thelium of varying thickness with numerous blood capillaries (which are limited by a basal lamina) embedded within it It usually covers the whole lateral wall between the prom nent a sp ralis and Reissner membrane but there may be areas of extensive atrophy in all coils in ears with good hearing f r all tones (Guild, 1935) Th early literature on the morphology of the stria vascularis is reviewed by Nachlas and Lurie (1951) Electron microscopic tudies in clude those of Engström, Sjöstrand and Spoendlin (1955) Smith (195) Rodriguez Echandia and Burgos (1965) Hinojosa and Rodriguez-Echandia (1966) and Spoendlin (196) These investigat rs ha e hown morphological evidence wh ch supports the theory that producti n of endolymph occurs in the stria vascularis. It is also believed to be the source of th positive 80 mV endolymphat c DC potential (Davi *et al* 1958; Misrahy *et al.*, 1958 and Tasaki and Spyropoulus 1959)



F g 1 Axial section through the cochlear duct of five-month human fetus half coil from the basal end. There close resemblance to the adult cochlea although in some respect full maturi y has not been reached For instance the mesenchymal layer under the basila membrane is rather thick. The sulcus internus (rrow) is not yet f lly dev loped and the inner ridge (see Fig. 20) is st ll discernible The stria vascularis las bows lpse of imma urit Osmic acid fixa ion, paraphenylendiamine staining, epon embedding. (Magnification 150)

The sensory epithelium of the cochlea, *the organ of Corti or the acoustic papilla*, is attached to the upper surface of the basilar membrane. It consists of an epithelial ridge about 100 μ thick which runs throughout the entire length of the cochlear duct. Its complicated structure contains two types of cells: supporting cells which form a firm but flexible framework, and hair cells, neuro-epithelial cells, which are assumed to function as transducers for sound energy to nerve impulses.

The supporting cells are slender cells which extend from the basilar membrane to the surface of the organ of Corti, where they form the rigid reticular membrane. The upper ends of the hair cells are suspended in this membrane in spirally running rows.

In a radial section of the organ of Corti (Fig. 1) a conspicuous structure is the tunnel of Corti, the pillar cells bounding it. Medially, close to the inner pillar, *the inner hair cells are surrounded by inner phalangeal cells and border cells*. The outer hair cells and the phalangeal or Deiters' cells form 3—5 rows, lateral to the tunnel and the space of Nuel. The outmost part of the organ of Corti is formed by Hensen cells. Nuel's space, between the outer hair cells and pillar cells, is one of the inter-cellular spaces, all of which, including the tunnel of Corti, communicate freely with one another. These are in fact one continuous space and it is only in radial section that they appear to be divided into several compartments. This intra-epithelical space is filled with a fluid, the cortilymph (Engström 1960 a). Thus the cell bodies of the outer hair cells are to a large extent surrounded by this fluid.

When studied from above the surface of the organ of Corti has a characteristic pattern which was well illustrated by Retzius (1884). Fig. 8 shows one of his figures of the reticular membrane.

The complicated system of supporting cells will not be described in detail. The reader is referred to the light microscopic studies of Retzius (1884), Held (1926) and Kolmer (192) and to the electron microscopic description by Iurato (196 196).

The hair cells are arranged in a characteristic pattern in the organ of Corti. This arrangement was the subject of an excellent description by Retzius (1884). Because of its pertinence to the present investigation it is desirable to review Retzius' description in some detail. In the basal coil the outer hair cells are arranged in three regular rows, an arrangement which is in complete agreement with that in the corresponding region of other mammals. A few single sensory cells are missing from the regular mosaic of cells and in such places the phalangeal processes of Deiters' cells lie close to each other, filling what otherwise would be a gap. There appears to be a certain irregularity of the pattern in the middle coil which is more pronounced in the upper part of the coil, where a fourth row of hair cells is sometimes found. The irregularity of the third row may also be rather pronounced and its relationship with the fourth row may be so complex that it is sometimes difficult to tell which cells belong to the third or to the fourth row. The latter tend to be discontinuous. Occasional sensory cells may be missing in the first, second [illegible]

and th rd row In the apical co l the irregularity of the third and fourth rows may be more pronounced The fourth row is regularly seen, but the second and the third rows are often incomplete Another type of irregularity seen occasionally in th middle and apical coils is the occurrence of a few s ngle cells wh ch are often disposed in the periphery of the reticular membrane thus form ng something wh ch resembles the beginning of a *fifth row of cells* Retzius remarks that there is a wide range of variat on in the pattern of sensory cells in d fferent ind viduals. The inner hair cells form a s ngle row of cells though a few supernumerary cells may occur close to and inward from, the standard single row

The prin ipal f atures of the structure of the hair cells have long been kn wn, but a cons derable quantity of new information has been added by many electron microscop c stud es in experimental anim ls during the last decade (Engström and Wersäll, 1953 a 1958 Smith and Dempsey 195" Engstrom 1960 b, 196 Iurato 1961 Spoendlin, 1966 Wersäll and Flock 196 and others) The outer hair cells are cylindrical n shape w th the nucleus in th lowe part of the cell. The upper cell-surface provided with a cuticle is suspended n an open ng in the reticular m mbrane The lower end is rounded and rests against a cup-shaped depress on n the Deiters cells A considerable part of the cell surface s n d rect contact with the intercellula fluid

Th inner ha r cells are pear-shaped, w th a relati el large cell body The cells apart from the upper surface wh ch is pro ded with a cuti le are surrounded b supporting cells The basal ends of both nner and outer ha r cells are n conta t with nerv end ngs A triking f ature n the human organ of Corti is the presence of large membrane limited inclusions in the ap cal zone of the sensory cells, p llar cells H nsen cells Deiters cells and sulcus cells (Kimura Schuknecht and Sand 1964) These granules ha e the histo hem cal charact ristics of lipofuscin (Iish i *et al.*, 196)

The cut cula area f the upper free urface of th sensory cells i prov ded with sensory ha rs, stereocilia arranged in a typical W-arrangement with the base f the W d rected outwards t t wards the piral ligament Interest in the function l s gnificance of the sensory ha rs w th respect to stimulati n of th rgan of Cort has mad th m the subject of several th rough ultra truc tural n estigations n lud ng those by Engstrom Ades and Hawkins (196) Kimu a Schuknecht and Sando (1961) W rs ll, Flock and Lundquist (1965) K mura (1966) Spoendl n (1966) and Wersall and Flock (196) Kimura Schuknecht and Sando (1964) have est mated the numbe of hairs on the surfaces of lim ted number of hai cells n the hum n cochlea On the ute sensory cells the ha rs were rranged n 6— parallel row th t tal numbers ang ng from 1 0—148 in the basal coil, nd f om 48—80 in the apical co l. On the nn sensory cell the ha rs were arranged n 3 to 4 row in a wavy f rm Th numbe of hairs n th basal coil wa about 48 A in most other dult mammals a basal bod but no k nocilium, is found on the sensory cells n th adult human cochl a

The available information on the numbers of sensory hair cells in the cochlea of man dates from the latter part of the 19th century Waldeyer (18.2) estimated the number of outer hair cells at 18 000 Krause (18 6) estimated 19,800; and Retzius (1884) 12 000 The same authors estimated the number of inner hair cells at 3,300 3 630 and 3,500 respectively No new information has appeared since

Several investigators have made calculations of the length of the acoustic papilla Retzius (1881) on the basis of measurements on three microdissected cochleas, indicated the average length to be 33.5 mm Hardy (1938) made calculations from 68 temporal bones sectioned and grahpically reconstructed according to the method of Guild (1921) and Crowe *et al* (1934) She reported the average total length of the acoustic papillae to be 31.5 mm with a range of slightly more than 10 mm the longest being 35.5 mm the shortest 25.3 mm a difference of about 40 per cent Hardy found no difference that she considered significant between right and left ear male or female nor between races.

The surface of the organ of Corti is covered by a jelly like filamentous structure *the tectorial membrane* which originates from the spiral limbus and extends over the hair cells to the Hensen cells. The longest hairs on each outer cell are in contact with the membrane (Kimura 1966) The free border of the tectorial membrane is fringed forming a network the Randfasernetz which according to de Vries (1949) and Tonndorf, Duvall and Reneau (196.) i in contact with the Hensen cells

The cochlea is innervated by both afferent and efferent nerve fibers It is also supplied with a system of autonomic nerve fibers The course of the nerve fibers innervating the cochlea is extremely complex and great problems are en countered in mapping and clarifying the cochlear innervation The investiga tions in this field are numerous, some of the most exhaustive studies having been reported by Retzius (1881 1894) Held (189 19 6) v Ebner (190.) Cajal (1909—1911) Lorente de No (1926 1933 a b 1937) and Fernandez (1951) Bocca (1954) has thoroughly reviewed this literature More recent investigations have been made by Rasmussen (1953 1960) Rossi and Cor tesina (1962, 1963 1965) Engstrom Ades and Andersson (1966) and Spoend lin (1966)

The first order neurons of *the afferent n rve fibers* have their ganglion cell in the spiral ganglion The bipolar gangl on cells are distrib uted throughout Rosenthal s canal in the modiolus. The peripheral dendrite end as a series of small and sparsely-granulated afferent nerve endings at the hair cell in the organ of Corti The nerve fibers are unmyelineated a they run within the organ of Cort becoming myelinated only as they p through the region of the l benula perforat The dendrites making contact with the inner hair cell ha mainly radial course while the dendrites associated with the nerve en l ngs on the out r hair cell have a spiral course of varying unknown di tance i the out r piral bun lles, within the organ of Corti (Held 19 6 Lorent d N 193 F rn ndez 19 1) The fibers from several afferent n rve

end ngs and from several outer hair cells are connected to a single dendrite The pattern f innervation in the organ of Corti is xtremely complex and is as yet far from clarification Spoendlin (1966) estimated n the cat, that the majority of the afferent nerves end on inner hair cells, only about one tenth extend ng to the outer ha r cells

From the habenula perforata the myelinated dendrites form bundles which run n canals through the osseous p ral lamina before they reach th spiral ganglion In the basal coil th course of the nerve bundles runs radially towards th modiolus but in the middle and ap cal coils, the bundles deviate obliquely basalwards t the piral gangl on, because the latter is short r than the organ of Corti and does not extend to the apex The innervation density as m asured by the concentration of ganglion cells, is greatest in the upper basal and l wer middle co ls There is a definite decrease in density of innervation at e th r end of the cochlea (Gu ld *et al.*, 1931 Wever 1949)

The ax ns of the gangl on cells pass centrally via small canals in the modiolus to the internal acoustic meatus where they converge to form the cochlear nerve They t rminate in synaptic contact with the cells of th cochlear nuclei in the medulla oblongata The afferent nerve fibers from the organ of Corti hav a tonotopic distribut on in the spiral gangli n, in the cochlear nerve and in the cochlea nuclei (Cajal, 1909—1911 Lorente de Nó 1933 a; Schuknecht and Woelln r 1953 1955; Sando 1965) The apical co l is represented in the center of the cochlear nerv The f bers initially pass in a piral around the cochlear axis, a tend ncy which cont nues even nto th free course of the nerve The lower coils are represented n the periphery and the f bers ha e a less tightly piralling course in the nerve

Th average numbe of nerve fibers in the human acoustic nerve is calculated to be 31 000 (23 000—40,000) (A T Rasmussen 1940) and the f ber diameters v ry from 1 to 10 μ (Engstr m and Rexed 1940; A. T Rasmussen, 1940) Wever (1949) ha calculated the a erage number of gangl on cells in th spiral ganglion to be 30,500

The eff rent innervation of the cochlea has its origin from the contralateral accessory oli ary nucleus (G L. Rasmussen, 1946 1953) from the homolateral main superi ol ry nucleus (G L. Rasmussen, 1960) and from the r ticula f rmati n (at least in the rabb t) (Rossi and Cortesina 196_, 1963) Th efferent bundle ent rs the cochle ia th olivo-cochlear anastomosis (Oort anast mosis) and runs in Rosenthal s canal, forming intragangli nl spiral bundles passes through the osse us spiral lamina in a spiral course It ends as th granulated nerve end ngs on both inner and out hair cells in the o gan f Corti (Iurat 196 ; Kimura and Wersall, 196 ; Spoendlin and G cek 1963 Smith and Rasmussen, 1963)

Rasmussen (1960) has lculated the number of fibers in the efferent bundle f th at to be approximately 500 Frequent branching of efferent nerves in th osseous piral lamina has been reported (Nomura and Schuknecht, 1965; Ish i Murakami and Balogh, 196) and the number of fibers in the habenula perf rata i calculated to be at least _ 80 (N mura and Schuknecht, 1965)

Spoendlin (1966) estimated the number of efferent fibers crossing the tunnel of Corti in the cat, to be around 8000 and the number of granulated nerve endings about 40 000.

In the human there are few studies on the distribution of the efferent innervation in the cochlea. Gacek (1961) reported spirally running fibers in the osseous spiral lamina as probably being of efferent nature. This description has been verified by aid of an acetylcholinesterase staining technique (Nomura and Schuknecht 1965; Nomura and Kirikae 1967 and Ishii, Murakami and Balogh, 1967). In the basal coil the spiral fascicles were thin and numerous near the base but they gradually thickened and diminished in number towards the middle turn. In addition to the spiral fibers, Ishii, Murakami and Balogh (1967) found fine radial fibers with acetylcholinesterase activity. In the basal coil these fibers seemed to reach the habenula perforata directly without a spiral course. In the cochlear nerve there were also found scattered fine fibers with acetylcholinesterase activity. This observation coincides with that of Gacek, Nomura and Balogh (1965) in the cat.

The extensive literature regarding the *autonomic innervation* of the cochlea includes the publications by Cajal (1909—1911), Lorente de Nó (1926, 193[illegible]), Agazzi (1945) and Andrzejewski (1955, 1956). During the last years the Falck—Hillarp histochemical method for the detection of catecholamine has been applied for the study of the cochlea (Terayama, Holz and Beck, 1965, 1966; Spoendlin and Lichtensteiger 1966, 196[illegible]). More thorough reviews of the pertinent literature are found in the last mentioned papers.

It is suggested that the sympathetic innervation of the inner ear is derived from postganglionic fibers of the inferior cervical stellate ganglion or from the superior cervical ganglion (Lorente de Nó 1926; Terayama, Holz and Beck 1966, Spoendlin and Lichtensteiger 196[illegible]) or has its origin from the central nervous system (Andrzejewski 1955). The sympathetic nerve fibers are described to follow the vessels in the modiolus and the osseous spiral lamina but the small vessels in the cochlear duct is considered to be without autonomic innervation (Lorente de Nó 1937, Smith 1951, 1957; Engström, Sjöstrand and Spoendlin 1955, Terayama, Holz and Beck, 1966; Spoendlin and Lichtensteiger 196[illegible]). Engström, Ades and Andersson (1966) mentioned the possibility that certain fine nerve fibers within the organ of Corti may be autonomic.

D. THE COCHLEA IN OLD AGE AND THE PATHOLOGICALLY ALTERED COCHLEA

1. *The cochlea in old age*

The term presbycusis is defined as any change in hearing associated with the ageing process (Glorig and Davis, 1961; Glorig and Nixon, 196[illegible]). However, great difficulties are encountered in distinguishing cases of true presbycusis from cases in which extraneous factors have contributed to the onset of deafness in the [illegible]. These problems are emphasized by the investiga-

t ons of Rosen and collaborat rs (196 1961) on the Mabaans members of an isolated Sudanese tribe who li e in a very quiet environment and have nev r been exposed to the noise of an urbanized community When Rosen compared the hearing of members f this tribe with that of correspond ng age groups in Ameri a Germany and Egypt he found thei hearing t be substantially more acute in the old r age groups Glorig and Nix n (1960) con s d red noise to be a crit cal factor n the d fference in hearing m ari us *populati ns of comparable age group*

Bergman (1966) who took part n the invest gation f th Mabaans, has compared the best h arers (top 10 %) in th U S with those of the Mabaans and found no *significant difference w th age th ugh the poorest hearers* (bottom 10 %) n the American studies how more rap d decrement in h aring with geing than the poorest of the Mabaans Bergman concludes t appears from these data that a majo explanati n f the stab lity reported f r the Mabaans in hearing thresh lds w th ageing is f und in their marked population homogeneity

In an in estigation on the hearing of the Todas belonging to an isolat d tribe in Ind a, who l ke the Mabaans, live in a v ry silent environment, it was found that these people also sh wed bette hearing n old age than pe pl f the i lized western w rld (Kapur and Patt, 196) These two tribes are widely separated f om each th r and are compl tely isolated, hence t is h ghly mprobable that a common genetic fa t r could account for the good hearing Both tribes have low cholesterol levels n the blood a low incidence f cardio-vascula disease and no rise in blood pressure w th ageing. Any o all of these fact rs may ha e some bearing upon the preservation of good h aring in old age and this would tend to be upported by the study of Rosen nd Olin (1965) who found bett high-frequency hearing among young people n a populat on with low rate f coronary heart d sease and atherosel ro is, nd low blood ch lesterol, than among young people n a population wh h is hyperch lesterol m c and th roseler tic and has a h gh incidence of cor nary heart disease

Th re is, as yet but ncomplete understanding of th reason why the hea ng in old r person is bett preserved in some populat ns than in other *but on th ng that quit lear is that th term presbycusis has included* hith rto many cases n whi h the hearing loss ha been du n t nly to ge ng tself but lso to othe fa tors

Exam nat n f the literatur fails t pro id us with any worthwh le pic ture of th histopath l gy f true presbycusis It has been common to appro h th probl m by assuming that elderly hard-of hearing people suff r f m presbycus nd to describe th r inner ea w th that assumpt n in m n l ev n th ugh other path logical conditions may have been uperim posed This is an app ach well calculated to obscure the issues Hence a rich *ari ty f h t l gical* changes ha e been linked to the clinical cond tion of p esbycusi Th range f such changes ndicated by the f llowing amples

At ph of th organ f Corti

b Degeneration of the spiral ganglion and the nerves in the osseous spiral lamina
c Changes in the central nervous system
d Changes in the blood vessels
e Mechanical causes due to loss of elasticity of tissues in the cochlea and middle ear

Finally there are many instances, in the histological investigations of aged and pathologically altered human cochleas in which the results are in doubt because of apparent errors of judgment often based on technical inadequacy of the preparations that is in which the illustrations and descriptions indicate that the author has failed to avoid the great difficulties inherent in the histological study of the human cochlea

a *Atrophy of the organ of Corti*

In their study of presbycusis v Fieandt and Saxén (1937) described in 19 out of 33 cases a pronounced atrophy of the organ of Corti as well as of other epithelial structures in the cochlear duct Since they found the pronounced changes in the blood vessels of the cochlea they called this type of degeneration angiosclerotic degeneration of the inner ear The changes in the organ of Corti were found to be spread diffusely throughout all coils of the cochlea The organ of Corti was flattened and deformed the sensory cells were reduced in number and the supporting cells were twisted and fragmented In the most advanced cases a thin single layer of epithelium had replaced the organ of Corti This condition of the organ of Corti bears a close resemblance to Wittmaack s neuroepitheldegeneration and several more recent reports of similar findings (Fleischer 1956 Jørgensen 1964 and Hansen and Reske-Nielsen, 1965) Fleischer (1956) and Jörgensen (1964) were unable to correlate these changes with increasing age The possibility that the compression and flattening of the organ of Corti is an artefact has been proposed in several papers (Lange 193ˇ Fernandez, 1958; Hansen and Reske-Nielsen 1965; and others)

Degeneration in the basal coil of the organ of Corti in old age is described in several reports In out of ‗6 cases Fal nyi (1931) found an atrophy of the organ of Corti in the lower basal coil but a correlation with increasing age was not clearly established

Crowe Guild and Polvogt (1934) in their study on the pathology of high tone deafness identified a group of cases characterized by an abrupt high tone hearing loss in which the most striking change was an atrophy of the organ of Corti in the lower basal coil The degenerative process extended as high as 10 mm from the basal end and in some cases there was also an atrophy of the nerves within the corresponding region of the osseous spiral lamina The average age in this group was 4ˇ years and the degenerative changes did not show any clear tendency to increase with advancing age

Fleischer (19 6) reported a study of 100 temporal bones with a uniform distribution from the first to the ninth decade of life Amongst these in four

elderly subjects degenerative changes in th organ of Corti could not be ruled out as the structural correlate to presbyeusis

Schuknecht (1961) ha classif ed presbycusis into four types. One of these sensory presbycusis, is characterized by an atrophy of the organ of Cort and nerves n the basal end of the cochlea The change usually begins in middl age and progresses slowly Even at ad anced age the lesion is usually limited to a few millimeters of the basal end of th cochlea Schuknecht suggested that the primary locus f degen ration may be in the supporting cells of the organ of *Corti and that the degeneration f the n rves may be a secondary* phen menon This reason ng is based upon his own earli r f nding that ex perimentally induced damage to hair cells produces no nerve degeneration unless the damage is so severe as to nvol e d teriorat on of the supporting cells (Schuknecht 1953 b) He indicated that this type of presbycusis sh ws an abrupt high-tone h aring loss

The occurrence of a normal population of sensory cells n the organ of Corti n old age is not infrequently reported h wever Bredberg (196) indicated that in old age significant sensory cell losses occur throughout all coils of the cochlea The most pronounced loss of sensory cells occurs in the basal coil; h were degenerati n of sensory cells n the apical co l may often be found as well These findings will be presented and th roughly discussed in a late hapter of the present publ cation

b *Degenerat n of the p al gangl on and nerves n the osseous pi al lamina*

The most constant histopath logical finding in the cochlea in old age is an atrophy of the spiral ganglion and the nerves of the osseous spiral lam na in th basal coil (Habermann 1891 Bruhl 1905 Manasse 1906 Fabinyi, 1931 F eandt and S xén 193 Fleische 1956 Schuknecht, 1955 1964 Jorgensen, 1964 Hansen and Reske-Nielsen, 1965 and Makish ma, 196) The degen rati e changes are most pron unced at the basal end and diminish t wards the apex. The organ of Corti is described as normal or near-normal in several reports (Fabinyi 1931 Crowe Guild and Polvogt, 1934 v Fleandt nd Saxen, 1937 and Schuknecht, 1955 1964)

In 4 ut f 46 temporal bones with a gradual high ton loss Crowe Gu ld and Pol ogt (1934) f und, in the basal coil, a partial atrophy of the nerves in th seous spi al lamina The nerve atrophy extended beyond th 10 mm level in all but one case, while the organ of Cort sh wed les ons l mited t the lower —3 mm of the basal turn Crowe Guild and Polvogt f und it apparent that in gen ral a relationship exists between extent of n rv atrophy in the basal turn nd the degree of h gh-tone hearing loss

In old guinea pigs (2—4 years) a degenerati n of the piral ganglion in the p l co ls has been described (Covell and Rogers 19)

Schuknecht (1955 1964) discussed the degenerati n of the piral ganglion n the basal coil and he regarded it as part of th general reduction of neurons n the central nerv us y tem which begins as early as th third decad of lif Likewise there is some degenerati n in the central aud tory pathways.

Schuknecht called this type of hearing loss of old age neural presbycusis and he considered that it meets the audiological criterion of presbycusis which Pestalozza and Shore (1955) Goetzinger *et al* (1961) and Hinchcliffe (1962) have described Speech discrimination scores in such cases are often low without a parallel loss in pure tone thresholds a condition which has been termed phonemic regression by Gaeth (1948)

Sercer and Krmpotic (1958) claimed that with advancing age hyperostotic deposits could develop at the fundus of the internal auditory meatus. These deposits would narrow the openings for the nerves and thus exert a continuous pressure on the nerve fibers causing atrophy Degeneration of the spiral ganglion cells is assumed to occur secondary to that atrophy

c *Changes in the central nervous system*

For many years degenerative changes in the central nervous system have been regarded as one of the possible ways to account for presbycusis, a view which has been based on indirect evidence Degenerative changes in the central auditory pathways and centers have been suggested in cases where the hearing loss could not be explained at a cochlear level (Sporleder 1899 Crowe Guild and Polvogt 1934 v Fieandt and Saxen 1937 and Schuknecht, 1955 1964) Several clues to the localization of different forms of hearing defect have been given by audiological methods and it is generally accepted that some of these permit one to place the causative lesion central to the cochlea (Pestalozza and Shore 1955 Bocca 19 8; Goetzinger *et al*., 1961 Hinchcliffe 196* and others)

Histopathological evidence which support an hypothesis of a central causation of presbycusis has been gradually accumulating during recent years. Brody (1955) found that the cells in the superior temporal gyrus (the anatomical location of the auditory cortical projection area) are reduced by half during the period from 20 years to 5 years of age Matzker (1958) has described degenerative changes in the ganglion cells of the superior olivary complex in ageing individuals Kirikae Sato and Shintara (1964) studied 11 brain selected at random from aged people (68—8 years) and compared the findings with those in the brain of 15 young adults (0—30 years) The older group showed uniform atrophy and degeneration of ganglion cells in the ventral cochlear nucleus and the medial geniculate body and various degrees of degeneration in cells distributed amongst relatively normal cells in the superior olivary nucleus and the inferior colliculus

H nsen and Reske-Nielsen (1965) have studied the central auditory pathways and temporal bones in 1. elderly subjects of whom 10 were over 80 years of age The central auditory pathways showed severe degeneration The cochlea showed slight to moderate loss of spiral ganglion cells in the basal coil and in addition a flattening of the organ of Corti throughout the coil the latter finding being regarded as mainly due to artefact The authors believed that the [illegible] loss could be explained by the cochlear finding

but they a umed that th hearing loss for l w tones was exclus vely central in origin.

Mak shima (196) reported two cases with degenerations in th auditory cort x of the temporal l be the entral cochlear nucleus and the gl al part of the VIIIth nerve

d *Changes n the blood vessels*

Changes in the blood vessels of the cochlea have been reported to be cor related w th presbycusis Fab ny (1931) f und a relationship between high tone loss and the degree of cerebral atherosclerosis with changes in the essel of the nt rnal acoust c m atus Von F'eandt and Saxen (193) described a pronounced angiosclerosis in the cochlea vessels a be ng one of the major causes f presbycusis They found s mila hanges in the vessels of the kidney but not at the base of the skull

Jorgensen (1961) repo ted a clear relationship between PAS-positiv thi kenings in the cochlear capillary walls and increa ing age with arterio-scl rosis especially n th blood vessels at the base of th skull

A co relation has not been found between changes n the stria vascularis and degeneration in the rgan of Cort or p ral gangl on (Fabinyi 1931 Crowe, Gu ld and Polvogt 1934 Guild 1935 Jorgensen 1964 Schuknecht 1955 1964)

Schuknecht (1961) described on type of presbycusis metabolic presby cusis where th hist pathol gical f nding was of a partial atrophy of the tria vascularis n the apical half f th cochlea w th a normal-appearing organ of Corti and spiral gangli n The hearing l ss in those ases hould be alm t equal fo all frequencies and is cons dered by th uthor to be due to defects in the phy ical and ch mical processes b which energy is produced n th tria ascularis

e *Mechan al causes due to lo of elast city of tissues n the cochlea and middl ea*

Ma (1919) described a thickening and hyal nization of th ba ilar mem-brane as ause f presbycusis These cl anges were most ev dent n th basal o l wh re d posits of calcium salts sometimes appeared May suggested that n old ge there occu a t ffening of th ba ilar membrane analogous to the los of la ticity n th len in cases of presbyopia

C ll nd Rogers (195) nd Pestalozza *et al* (195) observed n old guin pigs a reducti n of the cochlea micr ph nics which wa gre ter than they could attribute to the hanges n the rgan f Corti, and they uggested that a ign ficant conductiv hearing loss might explain this discrepancy

Gl rig nd D vis (1961) described a f rm of progress ve h gh ton con du t hearing l ss which begin t dev lop during middle l fe They sug gested th t th s conduct presbycu i mu t be ttributed to a les n n the m ddl They lso suggested, as an explanation of presbycusi an inne

ear conductive impairment related to physical change in certain tissues of the cochlear partition A high tone conductive hearing loss was also reported in the Mabaans by Rosen *et al* (1964) Schuknecht (1964) supported the hypothesis of inner ear conductive hearing loss. It constitutes one of his four types of presbycusis and he names it mechanical presbycusis

2 *Cochlear pathology not related to ageing*

There are numerous published reports on the pathologically altered human cochlea The following paragraphs present a brief survey of cochlear pathology in man which does not attempt to give a full review of all the literature

It is well established that *intense acoustic stimuli skull trauma and surgical manipulations of the auditory ossicles* may result in a characteristic hearing loss which in mild cases, manifests itself as a loss in threshold sensitivity for pure tones at a frequency around 4000 cps More severe injury extends the spectrum of hearing loss to include higher and lower frequencies, but the maximum hearing loss is usually in the 4000 cps region Many reports have appeared of th rough investigations into the structural functional physiological and biochemical changes in the inner ear resulting from exposure to noise of experimental animals (Lurie Davis and Hawkins 1944; Ruedi and Furrer 1946; Lindquist Neff and Schuknecht 1954 Schuknecht and Davidson, 1956; Rüedi 195 Koide *et al.*, 1960; Perlman and Kimura 1962 and others)

Our knowledge of structural changes in the human cochlea secondary to acoustic trauma is very sparse Tl e first report to be found in the literature was made by Habermann (1890) who described the cochleas of a blacksmith whose occupation had exposed him to high intensity noise during his working life The most exten ive damage was found in the upper part of the lower basal coil where the organ of Corti, the nerves in the osseous spiral lamina and the spiral ganglion were all completely degenerated Some years later Habermann (1906) described five cases in which atrophy of the organ of Corti an l the nerves in the osseous spiral lamina of the lower basal coil was supposed to have occurred a a result of noise damage Guild *et al* (1931) described a case in which the audiogram showed 4000 cps dip and hist logical examination revealed a circumscribed partial to-total degeneration of outer hair cells in a region 6.8—10 mm from the basal end of the cochlea The nerves nd spiral ganglion were normal

Tl abrupt high-tone l ss a described by Crowe Guild and P lvogt (1934) i con idered to be closely related to the 4000 cps dip (Glorig and Davi 1961) Hi t patl ologically tl cases of abrupt high tone los were reported to be characterized l y an atrophy of the organ of Corti sometimes extending a far a 10 mm from the basal end of the cochlea Thi atropl y often l owed a patcl y di tril ution with a corresponding degeneration of th nerves (Crowe t al., 1934) How er in 51 cocl lea from the collection of t mporal bones at John Hopkin Hospital We er (191_) wa unable t

correlate th 4000 cps d p with any localized structural change in the cochlea. The results sh wed a somewhat higher degree of pathol gy of inner ear structures in cases in which large d p were present, but no region was denti fied a pecially responsible for the local depressi n

In agreement with Habermann (1890) and Guild *et al* (1931) Igarashi, Schuknecht and Myers (1964) described, in three cases, a localized degenera tion n the basal coil f llowing noise exposure and skull trauma In two of these cases the hearing loss was documented w th audiograms showing charac teristic high tone losses, most severe at 4000 cps The lesions, 5—12 mm in width, consisted of a partial degenerati n of outer hair cells th maximum injury occurring in the 10 to 12 mm region of the cochlea The nerves in the osseous spiral lam na and the spiral gangl on cells sh wed no damage n corresponding regi ns

A considerable number of *chemicals and drugs* have been shown to have toxi effects on th ear From animal experiments the structural damag caused by som of these drugs is fai ly well known These studies include re ports on the t xic effects of arsenic ac d mercury combinations quin ne sali-cylates nd tobacc poisoning

Several ntib otics of the trept myces group wh lst otherwise of clinical value ha e the unfortunat side-effect of causing severe damage to the inner ea These antib ot cs include strept mycin d hydrostreptom cin kanamy in and neomycin There are numerous reports regarding the ot tox c effects of these antibi t cs in animals Review of the literature are to be f und in pub-lications by Tybergh in (196) and Koh nen (1965)

There are few reports regarding structural changes in the human inner ea following th adminst ati n of drugs Graf (1951) described basal degenera-t on of cochlear ha r cells and xtensive destruct on of vestibular epithelium as an effect of streptomycin therapy for cases of tuberculous meningitis. The ffects f ot toxi damage caused in man b kanamycin have been reported s degenerativ changes in the organ of Corti most severe in the basal coil (Benitez, Schuknecht and Brandenbu g, 1962 Jørgensen and Schmidt, 196_) Th mildest change was a los of outer hai cells, followed progressiv ly by loss of inner hair cell and finall of supporting cells In one subject w th neomycin induced d afness, the degenerat e changes were found to be most severe in both apex and base th inne hair cells be ng the most extens vely damaged (Lindsay Proct and Work, 1960) In ne ther the kanamyc n no the neomycin nduced lesions of the human a were there found any histo-l g cal hanges in the estibula epith lia The piral ganglion was reported to be norm l in ppe rance n all except one case (Jørgensen and Schm dt, 196) In thi case however the degen ration might w ll have been due t previon n se exposure as manifested in the audiogram as a hig tone hearing loss

Many drugs given to pregnant women may have dam ging effects upon the f tus the newborn child Some of these drugs ha e an totoxic effect and h t l gical reports f th tructural hanges of th nn ear include those

caused by thalidomide (Miehlke 1964; Rosendal 1965) and chloroquine phosphate (Lindsay 1967)

Inner ear pathology following *viral labyrinthitis* has been reported in relatively few instances to date A varying degree of degeneration in the cochlear structures has been attributed to measles (morbilli) (Nager 190 Lindsay and Hemenway 1964) mumps (Lindsay Davey and Ward 1960) infection of the upper respiratory tract (Lindsay 196) and sudden deafness, considered to be of viral etiology (Schuknecht *et al.*, 1962) The occurrence of congenital deafness due to maternal rubella i well known and there are some reports which describe the histopathology of the inner ear in fetuses and children in such cases Lindsay *et al* (19 3) and Keleman (1966) have reported cases and reviewed the literature

Since the first description of the pathology of *Ménière s disease* by Hallpike and Cairns (1938) there has been a gradual increase in the number of rep rts concerning the pathology of this disease (Rollin 1940 Lindsay 1944 1960 Dix Hallpike and Hood 1948; Schuknecht 1953 b Lawrence and McCabe 1959; Kristensen 1961 Schuknecht, Benitez and Beekhuis, 1962 Hansen and Reske-Nielsen 1964 Altman and Kornfeld 1965 Lindsay Kohut and Sciarra 196) A finding almost invariably reported has been of hydrops of the endolymphatic spaces in varying degrees This hydrops may be found in the cochlear duct alone mo t markedly in the apical coil or it may extend to include the saccule and utricule Ruptures of Reissner s membrane an l ruptures or herniations of the walls of the saccule and the utricule have also been described

In spite of hearing loss and recruitment, the organ of Corti has been reported to be morphologically normal in many cases of Ménière s disease (Lindsay 1944 1960 Schuknecht 19 3 b Schuknecht Benitez and Beekhui 1962; Hansen and Reske-Nielsen 1964; Lindsay Kohut and Sciarra 1967) In the more dvanced cases, as defined by Schuknecht Benitez and Beekhui (196) the organ of Corti ha been described a pathological (Hallpike and Cairn 1938; Dix Hallpike and Ho d 1948 Lindsay and v Schultess 1958 Kri tensen 1961 Lindsay Kohut and Sciarra 1967) Atrophy of the spiral ganglion and the nerves in the osseou spiral lamina has been reported in a few cases (Lindsay and v Schultess 1958 Kristensen 1961)

In Meniere s disease the vestibular sensory epithelium and ganglion have been found to be morphologically normal by light microscopy; however Schuknecht Benitez and Beekhu (1962) have described local degeneration of sensory cell in the macula sacculi in one case Electron microscopic stud es hav been made on the vestibul r epithelium in case with Meniere s disease (Pi trantoni nd Iurat 1960; Litton and Lawrence 1961 Friedmann *et al.*, 1963 Ireland an l Fark h d 1963 Fri dmann Cawth rne and Bird 196) but the evalu ti n of the fin ling i difficult because of technical difficulties an l tl fact that so far no stu ly of normal ultra tructure of human vestibular pith lium i ailal le for comparison

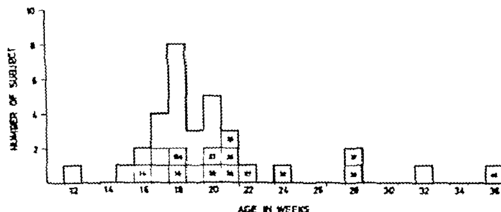
III MATERIAL

A PRENATAL

The basic material consisted of 58 cochleas from 9 fetuses however an additional 6 cochleas from 4 t llborn premature infants were also stud ed All the fetuses were obtained by transabdominal perations for legal abortion The age in weeks or luna months of each fetus was calculated fr m the f rst day f the last normal menstruati n In 15 instances the standing (crown heel) l ngth was measured and used for est mat on of age accord ng to Hamilton, Boyd and Mossman (196.) Tl e age distributi n is hown in F g. The sex of the fetuses was not noted

B POSTNATAL

The total mat rial ava lable included 119 cochleas from 4 subjects of which 53 were male and 21 female Their age distributi n is shown in Fig 3 A large number of the cochlea was used primarily for the development and improvement of the techn ques of preparation however many of these cochleas were also useful for other kinds of morpl olog ical study Applicat n of the f n l. perfected methodology was pos ibl n 41 cochleas from 2 subject .. male and 5 female, whose age d stribut n is ind cated in Fig. 3 It is this group which provides the majority of data for this tudy In 16 cases (8 cocl lea) all males, hearing tests we obtained prior to death



F g 2 Age distribution of the fetal nd premature material, consisting of 33 subjects. The small f gures thi the diagram represent the length (crown heel) of the fetuses and premature nfant from whom such nformat on was obtain ble Subject of 28 weeks or over re premature inf t

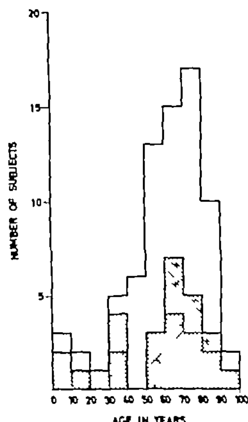


Fig 3 Age distribution of the post nat l mat rial f 4 subjects. The striped nd cross-hatched reas represent the 27 subjet whose cochleas were studied in detailed nd systematic way I 16 of these (represented by the cross-hatched rea) hearing test were allable The plain areas represent the materi l used f dev l pment f the technique nd f the m rphological studies.

C. DISCUSSION

The determin tion of the age of the fetuses presents a certain problem inasmuch as the time between onset of last menstruation and conception varies con iderably Confidence in the age determination is increased if the ize of the fetus is known In the present instance lengtl measurements could be ol tained from 15 but not from the remaining 14 of the fetuses for reason beyond the control of the author

The ge distribution of the fetuses is somewhat uneven with 2 falling between 15 and 2? weeks fetal age while only one was younger (1. weeks, 9 cm) and on older (21 weeks, 3 cm) as indicated in Fig . The reason for thi i of course related to the generally fortuitous character of human material whi h can only be obtained fter death All of the fetuses were obtained at tran bd minal operation for termination of pregnancy most of which are d ne between the 16th and 0th weeks Fetuses from early spontaneous or induced abortion were found to be macerated and showed such profound post m rtem leteriorati n a to be useless for our purposes Actually it wa n t the aim of tl i investigation to present a complete account of all a pect of cochle r developm nt l ut rather to estal lish tl e number nd p ttern of sensory cell in th f tus a a ba kgroun l f r studies n the young nd the adult human cochl a

The age distribution of the cochleas from young and adult postnatal subjects was also quite uneven the basic reason being of course the lower mortality rate in childhood and adolescence The volume of material is therefore weighted toward the middle to late decades of life It is only by a continuation of such a study as this over many years that the informational gaps in the younger age groups could be filled

Still another limitation of the material appears when we try to correlate hearing data with the morphological status of the cochlea Obviously only those subjects on whom ante mortem hearing tests have been obtained are of full value for this purpose *The initial aim of this study was to determine* whether or not the surface specimen technique (Engström Ades and Andersson 1966) could be adapted to the study of the human cochlea It soon became apparent that this was, indeed, quite feasible and as soon as the method had developed into a working routine the choice of subjects began *to incline toward those whose hearing had been* tested. Thus out of a *total* of 119 cases 41 were studied systematically in detail including 8 whose ante mortem hearing status had been assessed The number of subjects from whom such data can be assembled is determined purely by time and organization. Inasmuch as the primary purpose was to describe general principles in the technique of study and in the histopathology of the organ of Corti and associated nerves rather than to give a statistical analysis of the findings on a large number of tested ears, the number of cochleas studied was restricted

The preponderance of males ([illegible] out of 2[illegible] cases) is explained mainly by the fact that most of the patients whose hearing was tested suffered from pulmonary carcinoma a disease which occurs more frequently among males than among females No available tested ear was excluded The untested subjects were chosen according to age so as to make the age distribution as even as possible

IV METHODS

A SURFACE SPECIMEN TECHNIQUE

The basic method used for the anatomical study of the cochlea was the surface specimen technique developed by Engstrom and co-workers (196? 1961 1966) A full description of this method is given in the monograph by Engstrom Ades and Andersson (1966) Brief descriptions of the application of this technique to human subjects are found in papers by Bredberg Engstrom and Ades 1965 and Bredberg 1967 More detailed discussion of the method used in humans will be found in the following paragraphs

The young and the adult cochlea Within 10 hours of a patient s death the posterior half of the tympanic membrane was reflected forwards via an endaural approach through the outer ear canal thus exposing the posterior part of the middle ear cavity In order to expose the o al window region, it was sometimes neces ary to widen the postero-superior wall of the deep bony canal with a curet The incudostapedial joint was cut and the incus pushed aside to widen the operative field After cutting tl e tendon of the stapedius muscle the stapes was extracted The round window membrane was then perforated and partly removed with the aid of a small hook. When perforating the round window meml rane the greatest care was taken to avoid damage to the most basal part of the organ of Corti and osseous spiral lamina Posteriorly the round window membrane is separated from the osseous spiral lamina and the basilar membrane by a distance of only a few tenths of a millimeter Tl e hook used for perforating the meml rane was directed forwards, and parallel to the surface of the prom ntorial wall thus avoiding deep penetration of the scala tympani Using a glass micro-pipette *the fixative (ice-cold veron l buffered 1.5 % osmic acid solution)* was injected through the round window Thi injecti n wa made very carefully to minimize pressure changes within the cochlea When the injection began movement of tl e fluid surface in the oval window wa ea ily seen indicating a communication through the cochlea The fix tive thu perfused the cochlea through the scala tympani to the heli cotrema and through the scala vestil uli to the vestibule The mid lle ear wa filled up vi the oval window during the injection With another micro-pipette the fix tive wa removed from the middle ear and tl e injection procedure wa repeated 5 to 6 times with 0.5 to 1 ml of fixative each time Tl e middle ear wa then filled with filter paper ake l with fixative and the ear canal se led off with melted wax, whi h formed a tight plug a it congealed pre enting leakag of the fixative The whole procedure for exploring the middle ear and perfu ing tl e coel lea wa c rried out un ler an operating microscope

Sever l cochlea were fixed at system tically v ried time intervals aft r deatl in order to l termin to wh t extent po t mortem changes influenced th preserv ti n of urf ce pecimen Aft r 10 h ur the reti ul r membrane

st ll sh wed good preservation, the pattern of the cells be ng distinct and clearly isible in light microscopy Good results could sometimes be obtained in cochleas f xed after 15 hours or even later but n others the structures within the organ of Corti were n t dist nct, and evaluation of the pattern of the reticular membrane wa difficult however the reticular membran seems to be more resistant to post mortem changes than oth r elements of the organ of Corti The routine finally decided upon was to hold the post mortem fixation within 10 hours for surf ce preparati ns

The fixati e was left in the labyrinth until an autopsy could be performed. This was usually carried out with n one or two day The petrous portion of the temporal bone was removed from the skull with the aid of a chisel or a bone saw carefully avoiding damage to the promontorial wall and the labyrinth Immediately after removal of the petrous portion of the temporal bone the f xative was washed away by perfusing the labyrinth w th physiological saline solut on During this procedure the bone around the labyrinth was trimmed with a st ong bone-cutting forceps and, with the specimen mmersed in 0 % alcohol, the bony capsule around the cochlea was further thinned with dental diamond discs and diamond burrs until a thin bony shell was left The osmic acid f xati n darkens the membranous labyrinth, making it easily vis ble through the bony shell From this point on the membranous labyrinth can be exposed under direct isual control

W th the aid of fine needles, hooks and small watchmaker s forceps the remaining bony sh ll was broken and removed Exposure of the membranous cochlea was alway tarted at the apex, and then progressi ely extended towards the base Careful dissection at this stage made it possible to leave in tact th spiral ligament and associated basila and Reissner membranes. It is mportant to leave the cochl a duct unopened as long as possible because of tl e risk of contaminati n w th bone dust In the apical and middle coils it is usually ea y to remov the bony sh ll without damaging the cochlea duct however n the basal coils, and especially in its most basal portion, where the basila membrane is ery narr w and where a secondary osseous piral lam na is found, it is often best to leave the bony sh ll below the level of the basilar membrane which otherwise m ght rupture in an uncontrolled manner and thus damage the organ of Corti

The spiral l gament th tria vascularis and the Reissner membrane were emoved completely xposing the organ of Corti and the osse us spiral la mina (F gs 4 and 5) At this point macroscopic inspection of the organ of Cort a w ll as of the nerves in th osseous spiral lamina throughout all coils be omes poss ble, and photographs were taken of the cochlea a procedure which is described in a lat paragraph of this hapter It is practical at this stage t st re the cochlea in 0 % alcoh l, in the refrigerator if it is not possible f any reason to continue th dissection The final dissection may then be d ne at later time In such a case the pecimen is brought into distilled water by progressive dilution

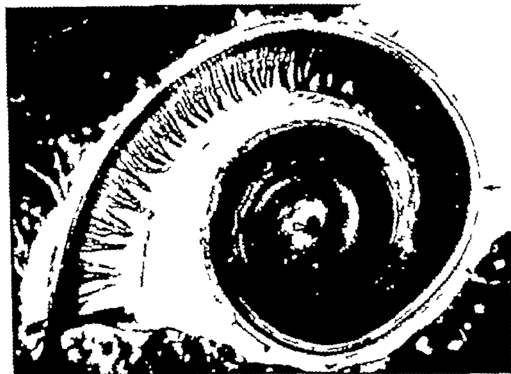
With the aid of a watchmaker s f rceps the tectorial membrane was tripped



Fig 4 Ad lt h m cochlea showi g the modi lus with the osseous spiral l mina d m ell ted nerv bundles. The bony capsul the spiral ligament nd Reissner membrane ha been remo ed The basal end f the osseous spiral l mi l seen th right nd the *low basal coil tends t the left si le of the photograph Abov it the middle nd pical* coil re exposed The cochl aqueduct (rrow) i sho l ing opened (M gnificatio 13)

of all coil (often in one piece) and mount d on a lide in glycerine f r further tudy under the phase contra t microscope The organ of Corti an l a sociated o seou piral lamina were cut in a radial direction at 10 defined level of the cochlea u ing a sharp pair of eye sci sors The organ of Corti wa thu livided into 11 separate segments which were remove l l y fracturing the osseou piral lamina cl se to the mo li lus The specimen were mounted in gl cerin on a gla slide with the l air bearing surface of the organ f Corti upward A co er-glass wa place l over the peeimen and these were tudied l phase contra t or conventional l ght microscopy

The divi i n of the org n of Corti into 11 segments wa done in a tan lar l-ized mann r Tl cochle wa viewed from above under the preparati n mi roscope The l rection of the mod olu wa a lju ted in the vertical plane in uch a w y that the helicotrem wa projected in the center of the two api al coil (Figs nd 6) Thu the co hlea i seen projecte l on to a plan perpen l cul r to the cochlear ax In th i way ev ry cochlea could be placed in a tan l rd posit n Into one e e piece of the mi roscope wa placed a gl pl t designed a a protractor m a uring 360 degrees Tl e cochle wa placed so tl t th cent of tl protractor wa projected o er tl center f th h licotrema and th z ro-l ne wa a tangent to the basal en l of tl osseou p ral lamina Tl angl between tl ro-lin and a line connect ng



F g. 5 Human cochlea seen from bov . Il eliasted nerv bundles within the osseous spiral lamina re seen ll i the low basal coil. The rgan of Corti (black arrow) is seen l ng t the peripheral margin of the osseous spiral lamina. The helicotrema i indicated with white rrow (Magnification 13)

a point n the organ of Corti and the hel cotrema represents the angle measurem nt of that particular point Thus half a co l from th basal end corresponds t 180 ne co l t 360 and two co ls to .0 (see Fig 6)

Th cuts separating th rgan of Corti nto 11 p eces were mad at the foll wing angles 1 30 60 90 135 180 2 0 360 510 and 0 In 15 cochl the actual distance n mm from the basal end t the diff rent angles was measured (Tabl 1) Th a erage distance from th basal nd f r e ch angl is hown in Fig 6) Th av rage length f the segments wa about 3 mm n th lower basal oil (0 —180) increasing to about 4 mm n high co ls.

Th f tal cochlea As soon as possible aft r rem al the fetal skull was opened nd the dura freed from th temporal bon Th labyrinth was dissected free tl cochlea pen d at the apex and th t pes extracted thus all w ng tl f xati to perfuse th ent re cochlea. The cochlea was kept n tl f xati f on t two hou wl reaft r th capsule was remo ed with at hmake f rceps and hooks thus expos ng the cochlea duct The further

) Owing to rch cal erro the angle of 30 has been incorrectly made to correspond, the graphic i distance of 5 mm instead of 1 3 mm from the bas l end.

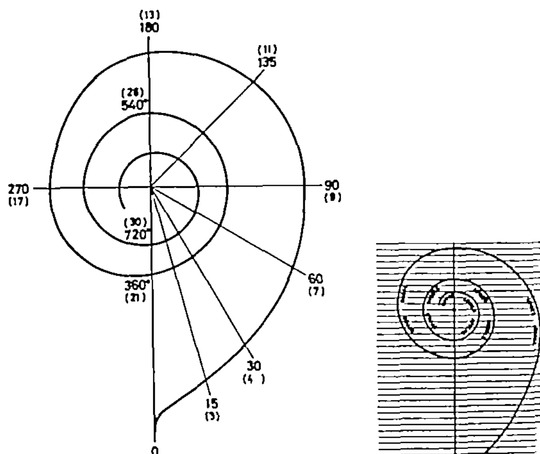


Fig 6 (Left) Drawi g based on photograph of the cochlea seen from bov (Fig. 5) The spirally curved line represent the rgan of Corti. This figure bow the locations of the d ff rent ngul measurement lo g the rg of Corti. F each of these measurement the rage distance from the basal end f the cochl l mm, l indicated between brackets. A distance of half coil from the basal end represent 180 of one coil 360 of one nd h lf coil 540 nd f two coil 7
(Right) Graphic reconstruction f the cochlea made from serial sectioning (Redrawn It H rdy 1938, Ame J Anat. 6.. 291—311) Thi figure illustrates the differences in interpreta tion f the term coil used i the sectioni g method nd l the present study Not that the line di iding the coil i t part lies t some distance from the basal end of the cochle Comp re this with the line i the left hand drawi g which t kes it poi t of ref rence from the basal extremity

preparat on wa c rried out by the procedure used f r the young and the adult cochl a The fixative used in mo t fetuses wa ice-cold veronal buffered 1.5 % o mic a i l soluti n

In a few cochl a a n rve staining meth d wa used Thi meth od wa de scribed by Maill t (1963) an l modified by Engstrom and Kohonen (1965) f r th stud of the cochle The fixati n-staining fluid con i t of two part a l eron l-l uff red osmi acid solution and a zinc iodide solution For d t led inf rmat regarding the use of th m thod see Kohonen (196 nd Eng tr m A l n l Anderson (1966) Th mode of preparation of tl l l a wa tl lescribe l abov with the l tin tl n that th

Table I. Different w of defining position along the organ of Corti. Measurements on 15 ad lt cochleas.

Angul measurement	Millimetre		Percentage of total length	
	Average	Range	Average	Range
	Distance from the basal end of the Cochlea			
15	3 1	1— 4.0	8.9	1—10.8
30	4 4	3.3— 5.2	12.8	11.0—14.2
60	6.	5.8— 4	19.4	1 .6— 1.3
90	8.	6.8— 9	25.1	22.3—?9.8
135	11.2	9 1—12.	33.2	30 1—36.
180	13.5	11 1—15.3	39.5	36.1—43.8
270	1 .3	15 4—19 9	50.8	46.5—51.3
360°	20.9	19 1—23.	61.5	58.1—65.5
540	.5.9	23.5—28.3	71.6	23—80 9
20	30 1	27.3—33 0	86.5	83.3—91.8
pe	34.0	29 9—3 .6	100.0	100.0

cochlea and the cochlear duct were opened w d l whil the specimen was immersed in the fixati n-staining flu d The spec men was kept in the solution about 3 hours. The final preparation was mad in the usual way but the pecimens w re dehydrated and leared in xyl l and finally mounted on a slide in Can da balsam

Several wh le cochl a   parts of them fixed w thin three hours of death, were embedded n epoxy res n (Epon) or acrylate prior t secti ning them f examinati n und   phase contrast or electron m roscopy Secti ns were made w th an LKB Ultrotom   and the electron microscope used was a Siemens Elmiscop 1 A. In the present in estigation these studies were used mainly to confirm observati ns made in surface pecim ns Further tudies on these mbedded pecimens are now in progres

B THE COCHLEOGRAM

The surface spec men tech nique m kes it possibl to surve th entire organ f Corti by xamination f the few segments nto wh h t ha been divided cco d ng t the procedure described abo   The ind dual sensory and supporting cells can be ident fied and localized in th reticular membran as hown by R tz us (1884) (Fig. 8) In most mammals (guinea pig rat, cat, abbit) th patt rn of sensory cells is of lmost geometric precision (Fig   ) This regularity makes t ea   to detect nd localize a place where a sensory cell is missing Comparison with corresponding areas in normal control cochl a thus makes possible an accurate estimate of th am unt of cell d generat n n a path l gical cochl a Degenerated sensory cells are f und nly rarely in normal oung animals hence in tud es of the effect of

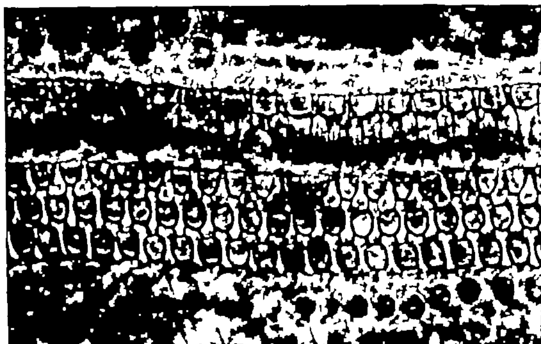


Fig. Surface preparation of th organ of Corti of guinea pig, bowi g three rows of outer hai cell (1 – 3) separated from ingle row of i ner hai cell (IHC) by the head of the *inner pilla cell* (P) Not the lmost *geometric regularity of the pattern.* (M gnifica-tion 80).

noxious agents on the inner ear the extent and pattern of lost cells and remaining intact cell can be mapped a a cochleogram (Engstrom Ades and Andersson 1966) A cell which i present i represented by the symbol o and the place of a lost cell i y ● The upper row represents the inner hair cell and the three lower rows, the outer hair cell (Fig 40)

The pattern of sensory cell in man a described by Retziu (1884) lacks the geometric regularity seen in lower mammal (Fig 8) For this reason deviation from a predictable pattern could not be depended upon a a way of d tecting degenerated or lost sensory cell in man a it can be in animals. Wl en a cell ha undergone d generation it disappears completely and the pl alangeal processes of the Deiters cell occupy it place forming a phalangeal scar (Eng trom Ades an l Andersson 1966) Tl us, although the sensory cell i lost the characteri tic appearance of the phalangeal processes makes it a y to i lentify and localize tl i los (Fig 9) Thi hold tru for the fir t three row of outer hair cells, where it i p ssible accordingly to g ve an accurate account of the ratio of intact to degenerated cell In th fourth an l fifth row wl le the intact cell present no prol lem it i difficult to detect w tl certaint tl number of cell l t because in tl periphery of the reticula m ml an tl e supporti g cell form an irregular arrangem nt which somet m m k o es n imit tes the phalange l scar

The ta k of g t re sonably precise estimat of tl e ext nt of sensory cell dam l ll mplified, a i tl presentati n of result

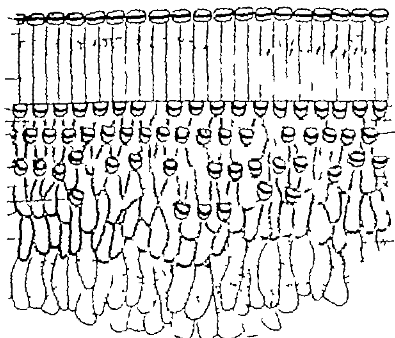


Fig. 8. Illustration made by Retzius in 1884, showing the surface of the organ of Corti, the middle coil in an adult man. This figure illustrates very well how accurately Retzius reproduced the surface pattern. From below downwards are seen a single row of inner hair cells, the heads of the outer pillars and three or four rows of outer hair cells, with the phalangeal processes of the Deiters cells between them. The positions of some missing outer hair cells are well shown. (From Retzius, 1884. Das Gehörorgan der Wirbeltiere II. Das Gehörorgan der Reptilien, der Vögel und der Säugetiere. Illustration XXXVII, Fig. 3.)



Fig. 9. Two stages of repair after degeneration of sensory cells as seen in surface specimens.

A. Collapse figure (arrow) indicating a recently degenerated outer hair cell.

B. Phalangeal scar which is the end stage of repair after degeneration of an outer hair cell. It indicates a healed-out lesion. (Magnification A and B 1410.)

if the classification of cells is confined to two categories. These are: (1) cell present or intact meaning that the cell is easily identified and its appearance is normal as seen in the surface specimen (2) cell degenerated meaning that it is absent or grossly changed in appearance In practical terms, this means that if the outlines of a sensory cell is seen but shows the collapse figure of relatively early degeneration or if it has been replaced by a phalangeal scar it is counted a degenerated (Fig 9) (See Engstrom Ades and Andersson 1966 for use of terms and more detailed account of the progression of hair cell degeneration as seen in surface specimens) The use of this sort of all-or-none classification may mi some details or more subtle transitional stages but in the light of previous studies in this laboratory thi would be more than offset by a reduction of the confusion which arises when one tries to classify all gradations of degeneration on the basis of criteria wh se validity may be dul lou

The cochleogram wa slightly modified in the mapping of the human organ of Corti The symbol of present (o) and lost (•) cells are the same In the three first rows both intact and absent cell are recorded but in the f urth and fifth rows only the intact cells In the first second and third rows a corrected pattern is reproduced i e a pattern in which all cells which seem to be referred to a certain row are recorded as being placed in that particular row When the pattern i adju ted in thi way much of the irregularity in these three rows disappears however some cells are *still distril uted irregu*larly and they are recorded in their true location on the cochleogram In the single row of inner hair cell the location of a single degenerated cell i ea ily detected however if there i extensive damage the exact number of absent cell may be difficult to estimate while the intact cells are ea ily seen Therefore in the cochleogram both intact and absent inner hair cell are recorded when degeneration i moderate but when extensive only the intact cells

A cochleogram con tructed according to the principles ju t described gives a recording of the arrangement of the sensory cells, which although not precise in showing the irregular arrangement, yield a fairly accurate estimate of the relation between intact and absent cell

The pos il ility of errors in the method due to different observers wa tested by the author and an experienced technician who independently con tructed cochleogram of identi al region at four level of five cochlea The segment counted wa 0 4 mm in length (the size of the cochleogram generally used in th tudy) Such a samp le contain in the intact cochlea on the averag 3—41 nner l air cell and 1 0—10 outer hair cell (th range of variati n lepen l on the difference in len ity between base and apex) In a t tal of 0 samples f m cochlea bowing a arying d gree of sensory cell degenera tion th m mum liff rence between the result obtained by the two observers wa ll both in the case of inn r and o t r hair cell In 11 of th samples of cell l in 11 of those of outer l ir cell the lifference n ver exceed

fct ntelery g

C. THE GRAPH OF CELLULAR POPULATIONS

The cochleogram gives detailed informati n about the pattern of sensory cells and intercellular relat ons, but t is not designed to express a survey f the whole sensory cell population therefore it became necessary to design a method whi h would present graphically th density of intact ha r cells throughout the cochlea The number of sensory cells present was counted from a cochleogram o by direct observation in the microscope in a segment representing 0 4 mm in length of the organ of Corti The density of cells was then calculated as the number of sensory cells per mm l ngth of the organ of Corti) In the lower basal coil (0 —180) a sample was counted about every second mm, in the uppe basal and middle co ls (180 — 20) about every fourth mm, and in the apical coil at a distance on and two mm from the apex In the average cochlea those levels represent the angle measurements of 15 30 60 90 135 180 . 0 360 540 20 (F g 6) The areas between the segments where cell c unts w re done w re surveyed unde the microscope to detect small areas which might show a deviati n from the state of degenerati n f und in the neighboring segments. When such a devi t on was observed, an extra sampl co ering this area was counted and recorded in the graph

The numbe of intact sensory cell per mm in ea h row of inner and outer ha r cells was calculated, as well a the total number f outer hair cells pe mm These number wer plotted against th distance from the basal end of the cochl of each sampl By connecting these po nts a graph was obt ined wh ch represented th correl ti n between d nsity of sensory cells and distance from the basal end of the ochlea As the length of the organ of Corti of man v ries, all cochlea were reduced to a common l ngth corresponding t the a rage lengtl of 34.0 mm

All m asurements of length were mad unde the microscope using a microm t scale as reference The length was measured at the line of contact be tween tl pilla heads and the first row of oute hair cell Fo measuring the tot l l ngth f the organ of Corti a f eld f i w of 0 40 mm was used and the specimen was m ved under the mi roscope fi ld by field, somewhat less than 100 times The err inherent in this manuevering was tested by two observers who independently measured the length of five cochleas The maximum difference n length measurements between the two observe w s 1.. %.

In 3 f th cochleas from which graph f the sensory cell populat on wer mad the total l ngth wa measured Th range of ariat n was between 30.3 mm nd 3 .6 mm and th av rage length was 34 0 mm

The t tal numbers of inner and of outer hai cells in a gi en cochl a w re lcul ted from the cell popul t n graphs (F gs 48—88) These numbers are p porti n l t the area en losed by th lin represent ng cell density and th ax o l n This are was measured with th aid f a planimeter and the

) I the present publication th defi tion of density of sensory cell used f not otherw se t ted in the text.

number of cells were calculated. As the length of the organ of Corti in the graphs was converted to the average length (31.0 mm) the number of sensory cells calculated as above mentioned also corresponds to the average cochlea. The actual number of sensory cells in each cochlea can easily be obtained (actual number = calculated number × $\frac{\text{actual length}}{\text{average length}}$). In three cochleas counts were made of every sensory cell directly under the microscope and corresponding numbers were also calculated from the graphs of these cochleas. These counts and calculations are shown in Table II. As seen from this table the two methods show close agreement. However, these cochleas showed intact or almost intact cell populations so that it is probable in cochleas showing a pronounced cell degeneration with variation in cell density that the agreement would not be as close. To make the error as small as possible the areas inbetween those where cell counts were made were surveyed in the microscope and circumscribed areas of degeneration were recorded in the graphs.

In order to evaluate to what extent sensory cell degeneration became more pronounced with increasing age, the total numbers of inner and outer hair cells of each cochlea were plotted against age (Fig. 93 and 94). In six cochleas the actual total numbers were impossible to estimate as the total length of the organ of Corti could not be measured because of slight preparation artefacts at the basal end. The numbers of cells in these cochleas were then calculated in reference to average length (31.0 mm).

To evaluate whether or not the degeneration related to ageing was evenly distributed throughout the cochlea, the number of sensory cells per mm was measured from the graphs of each cochlea in twelve regions distributed throughout the cochlea (15 30° 60 90 135° 2 0° 360 510 ... 0 Apex—2 mm Apex—1 mm). Measurements from corresponding levels of each cochlea were plotted against the age (Fig. 38). The slope of the distribution of the points in the diagram is proportional to the degeneration rate of sensory cells with increasing age. To be able to compare better the degeneration of inner and outer hair cells at different levels of the cochlea, the diagrams were constructed so that the density of cells of the completely intact population (average obtained from ... total cochl...) was plotted on the Y axis

Table II. Total cell counts obtained by direct counting and by calculation from the graphs of sensory cell populations.

Cochl. No.	Number of hair cells			
	Direct cell count		Calculated cell number	
	Inner	Outer	Inner	Outer
II 39 L (F ...)	[illegible]	16016	1130	15600
II 4 R (F ...)	[illegible]	11935	3123	11800
II 16 L (F ...)	[illegible]	[illegible]	3300	[illegible]

[illegible]

corresponding to an id ntical distance from the base l ne in all diagrams Accord ngly a given slope of each plotted populat on of counts corresponds to the same relative decre se of cells in ll diagrams From cons derat ons of space, only six such diagrams are illustrated in figures although all of them f rm the basis of the descript on giv n in the text

D Photography

The pictures of the wh le cochlea were obtained by ph tograph ng with a Hasselblad 500 C, 6×6 roll film cam ra equippe l with a Zeiss S-Planar 5,6/ 120 mm lens In most instances an extensi n tube 40 to 60 cm in length was used between amera and l n Th cochlea wa prepared accord ng to the technique described The bone below th basal co l was th nned out, lea ng only a thin hell The specimen was dehydrated n alcohol and immersed in xylol in a glass jar and photographed from a directi n perpend cula to the fluid surface The illumination was d rected mainly from bel w Two or three rd nary microscope lamps served as light source A s mple l ns (focus 10 cm) was used to focus the l ght on a small pot.

A problem was reated by the necessity of taking photographs through th surface of the fluid n wh ch the specimen wa mm rsed The surface was extremely sensitive t brat ons the occurrence of whi h spoiled many ex posures The solution of this problem depend d upon good illum nation and fast film emuls on, these two factors operat ng jointly to minimize xposure time Kodak Tri X Pan 120 film wa used and the exposure t me varied from 0.5 to 10 0 seconds The cam ra was f rmly fixed t reduce v brati n, which was further reduced by opening th shutt r in darkness and controlling ex posure by swit h ng the light ource on and off.

A second problem was that of depth f field which is min mized at the necessarily hort distance fr m lens to bject This can be partially compensated f by reduc ng the aperture as much as possibl howev r this ha the disad antage f reducing the resolving pow r of the lens and the exposure t m must be in reased correspondingly An optimal compromise between these sev ral facto s wa rea hed by using an perture f f/11 to f/16

The photomicrographs were taken on the Wild M 0 research m croscope pro ded with ph se contra t equipment nd camera using Kodak Tri-X Pan 1 0 film

E. Audiometry

H ring test were obtained from 16 subjects (8 rs) In of them (ear N s. H 83 R and L. H 85 L. H 90 R and L. H 93 R nd L. H 94 R and L. H 103 L. H 104 R and L) pure-tone audi metry was perf rmed in the wards a portabl ud meter quipped w th sound insulated h ad set be ng used for conduct n tests Conducti e components of hearing loss were ruled ut by R nne test using C_2 tun ng f k togeth with careful nspection of the

tympanic meml rane In addition mobility of the stapes was examined at autopsy The result indicated no evidence of any major conductive hearing lo

All other hearing tests were performed in a soundproof room at the Audiology Department using standard audiological equipment In 6 subjects (11 ears) speech audiometry was performed with determination of speech threshold and discrimination according to Lidén (1951) In 6 subjects (1 ears) continuou tone Békésy audiograms were taken with an audiometer built according to Bekesy s (1947) description The audiometers were calibrated according to the British standard

Time intervals between the hearing tests and the fixation of the cochlea were a follows

within one week 6 ears	(Nos. H 83 R and L, H 94 R and L, H 93 R and L);
1 week—1 month: 3 ears	(Nos H 42 R, H 85 L, H 103 L);
1—3 month : 9 ears	(Nos H 3_ R H 9_ R and L, H 98 R and L, H 108 R and L, H 109 R and L)
3—6 month : 6 ears	(Nos H 104 R and L, H 106 R and L, H 10 R and L);
1*—14 m nth 4 ears	(No H 90 R and L, H 110 R and L)

F DISCUSSION

Post mortem changes and artefact are regarded as great problems in the study of the human cochlea Fernandez (1958) found a con iderable variation in the preservation of inner ear structures among specimen for which the interval from death to fixation wa identical In specimens fixed by im mersion of th temporal bone in the fixative fluid a few hours after death, fairly advanced autolytic changes were occa ionally found Refrigeration an l intratympanal injection of formalin soon after death give better preservati n of the inner ear tructures (F rnandez, 1958 Schuknecht, 1968) The per fusi n technique used in the present study wa applied to further minimize tl e post mortem autolysi

The f ct r of p t mortem autoly i ha been stu lied in electron microscopy l y several investigators M ny hanges earlier interj reted a autolysi resulting from delayed fixati n were regarded by Ito (196_) a artefact of preparation After two h urs most of the fine features of the liver cell were almo t in l tingui hal le from those of freshly fixed tissue Hi preliminary studies on j an rea ki lney stomach and muscle in sev ral animal species pointed t w rd imil r conclu i n for tl ese ti ues

Electron m roscopy on the l uman organ of Corti (Kimura Schuknecht and S n lo 196) l owed f rly good preservation of th intracellular structures in specim f ed t l ours post mortem In tl e guinea pig Wers ll Ki mura an l l n lq t (196) les bed earl po t m rtem changes in spec m n f xed t l th Aft r 3 hours tl gen ral haracter of th

cell was still recognizable while after 6 hours the cell were mark dly distorted.

Several factors contributed t the preservation of the organ of Corti in the present tudy even when fixation was delayed fo 10—15 hours post mortem so that detailed mapp ng of sensory and supporting elements was still pos ble under l ght microscopy The primary facto wa perfus on as mentioned above A second factor is that th method is based on a stud of the patt rn of cells in the reticular membrane and th s seems to be more resistant to post mortem disintegration than other structural deta ls in the organ of Corti. Th s is also *the opinion of Johnsson and Hawkins (196) and t is supported by the ob*servat on that the reticular membrane forms a stiff plat (Békes 1953) which is not readily broken (Katsuki and Co ll 1953) Th surface specim n technique has the further ad antage that decalcification and embedd ng are av ided, and therefore el m nated as sources f additional artefacts

Fernandez (1958) described a post mortem change in th organ of Corti in 40 per cent of his own material which he called the compress on phenomen n and regarded a an artefact A similar change has also been reported frequently by others It consists of a gen ral compression of all structures in the organ of Cort nd of th sp ral limbus, often accompanied by a flattening of the tria vascularis These features match those described by Wittmaack (1924) unde hi term hypoton c degeneration and also those termed *endolymphati compressi n by Mygind (1945) both be ng ascribed to vital* processes The angiosclerotic degeneration of Fieandt and Saxén (193) wh ch was described in m re th n 50 per cent of their cases of presbycu is also shows a resemblance t the compressi n phenomenon In the present mat rial ***not a single cochlea out of 110 sh wed a mila compress on of the organ of Cort o the p ral l mbus*** The hape of th organ f Corti and the p ral limbus are easily bserved directly und th tereo-microsc pe and th height f the epithel um can be measured re d ly under phase contrast

When discussing the techn que pressure ariati ns appearing when the st pes extracted and the cochlea is perfused mu t be t ken into consideration Kimura Schuknecht and Sand (1964) used a similar technique for perfusing th cochlea and they d d n t attribute any ultra tructural artefacts th t hnique tself. Th t a fun t n ng cochl a can sust in th t tapedect mi has been h wn in numerous perations for tosel ... es the possib lity exists that the labyrinth n m mbranes may al f ... even rupture if th pressure changes were large Therefore the ... f th tapes nd th perfus on of the cochle were carried out ... und direct visual control in th perat n microscope

The nd l mph ti spaces were n t opened during the whole ... of f xati n in o der t min mize the h ft n pos t on of the membranes ... ting those parts Th positi ns f Reissn r membran and the ... in th estibula l byrinth re learly i ibl und the stereo... *during the preparati n f a specimen In n hum n cochl a* ... l ght d pr ess n of Reissner membran while in all the ...

fcts ... ...

was no notable deviation from it normal po ition In contrast to thi the well known depression of Rei ner s meml rane characteri tic of congenitally deaf Dalmatian dogs wa clearly seen in five such animal

In the present investigation no structures other than the organ of Corti and the nerves in the os eous spiral lamina were especially studied This limitation wa determined by the aim of the study rather than by limitations of the tech nique used The ba ic method i adaptable to the study of almo t any structure in the cochlea either by direct observation in the stereo microscope as surface specimen or a section made after embedding and sectioning of the appropriate portion of the cochlea Kellerhals *et al* (1967) for example ha made tudies on the spiral ganglion u ing a similar technique of preparation

An essential question in all histological tudies of human autopsy material i whether the disease which caused the death of the patient has had any influence upon the structures under study Thi problem will be discu sed in chapter VI

In the present investigation the sensory cells have been classified either a *present* or a completely degenerated or *lo t* Such an arbitrary classification of the cells into two group is, of course inexact inasmuch a the degeneration follows a gradual progres ion However from animal experiment the process of degeneration after exposure to noise or ototoxic drugs have been observed to occur within a very short period of time (hours or a few days) Fo thi reason it seem reasonal l to use the arl itrary all-or none categorie f r the present purpose It is possible of course that the human ears may how a imilar resp nse to such factors a drugs the patient s disease or the final agonal stage itself However the early stages of repair after degenerati n can ea ily be identified in a surface specimen (Fig 9) It hould also be emphasized that a normal appearance of a cell in light microscopic or even electron microscopi examination gives no a urance that the cell wa functioning n rmally before death alth ugh one u ually a umes tacitly that it wa from lack of more pro al le criteria

A prol lem whi h i crucial to a study of thi sort i that of id ntifying relative p ition on the organ of Corti In previou hi topathological studies of the human cochl a two y tem of t rminology ha e been used One of these employ t rm uel a lower basal coil upper l sal coil lower middle coil and so on sy tem wl ich at best afford no more than a rough localization Th second method, used in c njunction with graphic recon truction of th org n of Corti from seri l section attempt to l calize regi n in t rm of di tance in millimet r from th basal d of the cochlea The pri mary di adv ntage of thi method stem from th con i l ral l variation in t t l length of th org n of Corti from one i dividual to n th r (Hardy 1938) whi h m n that in two cochlea of diff ring l ngth identi al distan es m red from th base l not gnif th me rel tive p iti n n th ((l represe t different fra tion of the tot l length) Accord ng to B (D *et al* (1949) n l Schuknecht (1953a) th re i a n a l between th logarith m of frequ nc timulu an l the

distance from the basal end to the area of localization as measured in percentage of total length For that reason t should be more valid t compare locations along different organs f Corti in terms of relat ve rath r than absolute distance

In the present investigation a position along the organ of Corti was indicated either as the distance in millimeters o as angular measurements from the basal end of th cochlea One reason for using the angular measurements is that t gives a better topographical information regarding the position in the cochlea than distance as mm o as percentage of total length. In the graphs of cellular population the length wa corrected to a standard value so as to perm t intercomparison The angle correlat ons to d ff rent le els of the a erage coel le were used to con truct a scale f r the graphs, so that ea h represented the same proportion of total length n every cochl a In table I (page 39) are compared distances from the ba al end expressed as angular measurements as millimetre and as percentage of total length)

A furthe advantage of us ng angl measurements arises from the fact that in the course of dissecti n of a cochlea it i not always possibl to avo d a mall preparation art fact, especially in the lower basal coil In such an instance n ithe the t tal length n any po nt abo e tl area of destructi n an be accurately measured whereas use of the angle m asurement t ll permits defin ti n of the various po nts al ng the organ of Corti

It is mportant to emph ize that the measurements were made in such a way that the angl of 180 really ind cates ne half turn from the most basal end 360 corresponds to on full turn, 540 to one and a half turns, nd so on (Fig 6) In n ent onal serial section ng of human temporal bones, th cochle is divided into its component turns n ef rence to the plane of secti n, which is d fined in terms f the general topography f the temporal bone Thus the pl ne which di des th cochlea nto h lf-turns is dent fied a the plan of the m d-modiola h rizontal section (or n v rt cal section ng, as a mid mod ola plane at right angles to the sectioning pl ne) wh ch means that the distance between this plane nd th most basal end of the cochl a aries cons derably F r this eason the t m thods d not correspond xa tly n the use of th term coil (Fig 6) It seems, l ow ve more r l nt to use th t rm as describ ng a pos t on in reference to th basal end of the cochl a than n reference to the pl ne of sect oning.

The method used n this study t measure the l ngth of the organ of *Corti* ma conta n certa n poss bl rrors lthough th difference in mea *urements* m d ndepend ntly by two observers w small (1 2 percent of total *length*) On source of erro t be con idered is tl at the scissor cuts used t d *the* pecim n int segments may destr y a part of th rgan of Corti *large enough* t affect th me urem nts Thi was tested by m k ng oblique *at* the rgan f Cort It w f und th t the cutting procedure destr *y* ll correspond ng t di tance of 10— 0 (n length *Another possible*

) th ng te technical rror the angle of 30 ha been incorrectly made the graphs te distance of 3 mm instead of 4 t mm from the basal end

source of error would be that resulting from compression of the specimen by the cover glass tending to increase the linear measurement; however there is attached to each surface specimen of the organ of Corti its associated segment of the osseous spiral lamina which constitutes the thickest part of the specimen. The bone is not likely to change its form by the slight pressure of the cover glass and the organ of Corti is not under pressure.

The average length of the organ of Corti in the present material was 34.0 mm. This compares well with Retzius' (1884) value of 33.5 mm which, however, is an average from only three cochleas. Hardy (1938) found slightly smaller values, with an average of 31.5 mm in a series of 68 temporal bones, by measuring the length from a graphic reconstruction of serial sections. Hardy's graph is equivalent to an enlargement of an ortho-projection of the line of the heads of the pillar cells onto a plane surface at right angles to the modiolar axis of the cochlea. She did not correct for the error which arises from the fact that the cochlea has height. This error is composed of two factors, one corresponding to the gradual slope of the coils and the other dependent upon the most basal "hook" of the osseous spiral lamina (see Fig. 4). The first factor was considered by Hardy to be less than 1 %. The second factor was calculated in the present investigation from a top-view photograph of a cochlea and the angle-length measurement (Figs. 5 and 6). It was found to be about 1 mm. Thus, if corrected by these figures, Hardy's measurements of *the length of the organ of Corti are 1—1.5 mm shorter than those of the* present study. This difference is not large considering the difference in methodology.

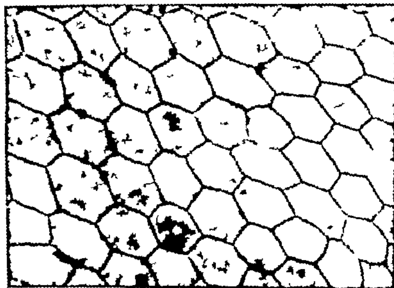
V RESULTS

A FETAL DEVELOPMENT OF THE ORGAN OF CORTI

1 *Cytoarchitecture*

The principal a m of the present study of the fetal cochlea was to trace *the development of the pattern of sensory and support ng elements of the* organ of Cort as part of the background f an in estigati n on the struc tural pattern of the young and the adult human cochlea Th tudy co ers the fetal ages from three to s x lunar m nths with some additi nal notes on the tatus at e ght months (premature ch ldren) It must be emphasized that the descriptions of the cochleas at the ages of three and s x months are based on the observat on of a s ngle fetus f each age Th s of cou se makes the f nd ngs less rel able as rep esentative f these part cular ages but when considered n relation to observat ons mad at intermediate ages it is evident that they pro ide valid contribut ons to th study of the process f development and differentiation

In the th ee months (9 cm) human fetus the cochlea has f rmed all its turns but h s not rea hed ts full size (The length of the cochlear duct was 0 mm) The scala vestibuli and tympani has begun to differentiate from the surr und ng mesenchyme the development of the f rmer being more ad anced than tl at of th latter The future organ of Corti consists of a thi k



F g 18 Surfac ew of the inne ridge low t the fut re organ of Corti of the m h hum fetus showing the uniform pattern of he cell The black dot seen on s ll surfaces represent kinocilia (M gnification 1 35)



Fig. 11. Three surface preparations of the rga of Cor ti f three-month huma f tus showi g different st ges of dev lopment t d ff rent lev l f the cochlea.
A. One cell (11 mm) from the basal end of the cochlea the i ne hai cell (IHC) ca be d ti guished by thei d rk ppearance. However there is as y t no ign of differentiation f the sensory nd pporting cell in the rea f the f t re outer hai cells (between rows).
B. I th region, 3—4 mm from the basal end, both inne (IHC) nd out (1 – 3) hai cell re diff rentiated.
C. 1 mm from the basal end, the surface rea of the sensory cells is smalle. The inne h i cells (IHC) re well seen, but some of the outer hai cell re difficult t distingui h from the supporti g cells. (M gnification, A 1l, nd C 1170)

ning of the epithelium which ha formed two spirally running ridges at the base of the cochlea. A seen from above in a surface specimen most of the epith lium show a haracteri tic unif rm pattern of polygonal cell surface (Fig. 10); however in a narrow regi n of the epithelium at the junction of th inner and outer ridges cell are organiz d in pirally running rows in dicating the region of inner an l out r hair cell. The first sign of differentiation a seen in osmium-fixed pecimen und r the phase contra t microscope i that the upper part of the sensory cell ha a dark r appearance than that f th upporting cell.

Three to f u mm from th basal en l of the cochl a the outer hair cell are li p sed in three regular row and ha e a roun l, free upper surface wh rea th I ir cell l e in ingl row an l present a larger free upper urf ce (l g 11 B). Th i r nd out r hair cell are separated l y the inn r and ou t l t th free urf ce of th epithelium how er the

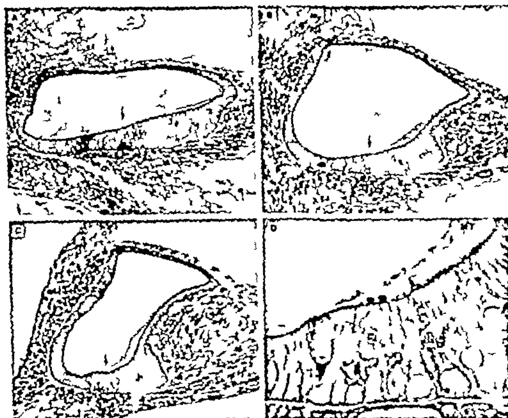


Fig. 12. Sections through the cochlear duct of three-month human fetus from three dif ferent levels of the cochlea. The epithel um of the cochlea duct stand out clearly from the mesenchymal tissue
A One and half coils (15 mm) from the basal end, the scal estibula is more clearly developed than the scala tympani. The future hai cell rea (indicated with arrow) is diffi cult to disti guish with certainty This section is light oblique so that the cochlea duct has more oval ppearance than is usu l on radial section.
B Three quarters of coil (9 mm) from the basal end, the hai cells re discernible (rrow).
C 3—4 mm from the basal end the hai cells (arrow) can be clearly differentiated (see D below).
D Same rea as in C (bov) highe magnif tion, showing one inne hai cell (IHC) and three outer hai cell (1 2, 3). Hairs can be seen on both inne and outer hai cells. N te the sclerocili (arrow) on the supporting cells unde the tectorial membrane (MT) which has begun to appea t ll lev l (Magnification, A B, and C 1 0 D 450)

ner pill r heads l ne are represented On the free surfaces of sensory cells well a supporting cells, a kinoc lium i present, isible already in the phase contra t microscope as distinct dark dot. Stereocilia are present on both an ad out r hair cell (Fig 12)

F m thi regi n towards the basal nd of the organ of Corti both inner nd t ha r cells become malle (Fig. 11 C) The inner hair cells arranged regul r w are distin t and clearly isible right t th end of the organ f C rt w rea the outer hai cell are irregularl arranged in t o or three

rows, and in the la t millimeter are indi tingui hal l from the upporting cells

Apicalward of the differentiated region a similar gradual change of pattern i seen Half a coil (7 mm) from the ba al end the outer hair cell are still regularly arranged and present a very small square free surface A little less than one coil (10 mm) from the basal end the arrangement becomes irregular and the hair cell are still smaller and difficult to di tingui h from the supporting cell The inner hair cell are still clearly vi il le although the size of the free surface i smaller (Fig 11 A) Apicalward at a point one and a half coil (1 mm) from the l ase n ither outer nor inner hair cell can be differentiated at the free surface of the epithelium The appearance of the urface i uniform; however deep focu ing reveals a regular row of cell nuclei below the line of junction between the inner and outer ridges which i discernible a a shallow groove in the epithelium The location of the nuclei correspond t the future location of the inner hair cells Within a few more millimeters apicalwards even thi ign of differentiation i no longer vi il le Intra epithelial fluid spaces were not observed at any level of the cochlea at thi fetal age

The mesenchymal plate beneath the cochlear duct i richly vascularized

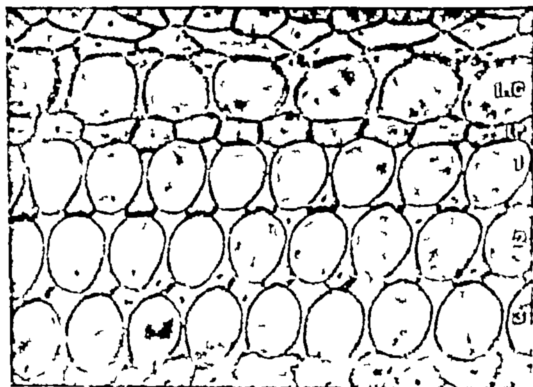


Fig 17 [illegible] rf [illegible] prepa [illegible] of he [illegible] f (rt half [illegible] 1 (11 mm) f om the basal end from [illegible] fou mon l h ma fetus [illegible] (IHC) [illegible] (1 – 3) [illegible] cell [illegible] well developed [illegible] he [illegible] ll pa t rn [illegible] [illegible] ral [illegible] k ne [illegible] [illegible] h [illegible] nd pport g ll (M gnific tion 1,00)

tct [illegible] g p; l



Fig. 14. Organ of Corti of a f e-month human fetus, surface preparation from the lower basal coil. The cell surfaces of the hair cells show mat form. The outer hair cells form three regular rows. (Magnification 18"5)

The most prominent vessel runs parallel below the junction of the two epithelial ridges (Fig. 12). In the basal coil it measured 0—40 μ in diameter. It is continuous from base to apex, though its diameter diminishes in the apical coil. Vessels from the modiolus run outwards to the spiral vessel, the latter sending branches towards the periphery. Some vessels run from the modiolus directly to the periphery without connection with the spiral vessel. Vessels crossing the future basilar membrane occur more frequently in the apical coil.

At four months (14—1 cm) both scala tympani and scala vestibuli are well developed and the organ of Corti has reached its mature length. As compared with the three-month fetus the surface pattern has changed considerably (Fig. 13). The inner hair cells can be distinguished throughout all coils of the cochlea. *In the basal one and one-half coils (0 —540) their free su*rfaces are large and oval. Further towards the apex they are more rounded and smaller. Occasional supernumerary inner hair cells are distributed throughout all coils.

Outer hair cells can be recognized from the basal end to within a few mm from the apex. In the basal coil the free surfaces of the outer hair cells are rounded and the cells arranged in three regular rows; however, in the most basal two mm they are distributed irregularly *in two or three rows*. *At a* distance of one coil (1 mm) from the basal end the cell surfaces become



Fig. 15. Orga f Corti of fi e-mo th huma f tus, surf ce preparation from the uppe middle coil. At this pe fou row of outer hai cells frequently occu i the middle nd pical coils. (Same f tus s Fig. 11) (M gnification 1875)

sligh tl maller a feature whi h i more pronounced two coil from the base. The size dimin hes still further in the lower apical coil and th cell are irregularly arrange l in two or three row Cl se to the apex th outer ha r cell cannot be diff rentiated from up porting cell

Alth ugh in g neral the pattern of out r sensory cell i very regular in the b sal an l m l ll coil occa ional irregul rities re present in mo t cases in the f rm of ngle upernum rary cell A f w cell forming a f urth row are occ i nally found At thi t g the tereocilia n the sensory cell display the l ra t ri t c W-shaped patt rn (Fig 16) The out r hair cell of th pical co l re th only except on to thi Fluid s ces in the rgan of Corti t ll not seen Th blood essel l w no t iking diff rence fr m th three-m nth t ge

It f m th f 3 cm) th free upper urf ces f th out r h ir cell f th l l m ddl co l ha acquired th gen ral l pe hara t ri ti of m ture t l l F g 11) h wev r n th p c l coil th r free ur

Acta otolar p

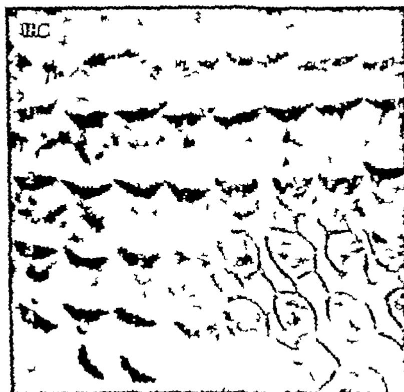


Fig. 14. Organ of Corti of .. month human fetus surface preparation from the upper middle coil, showing the arrangement of the sensory hairs. The characteristic W-arrangement is disturbed, seen on many of the outer hair cells (1, 2, 3, 4, 5). In the right lower corner of the microphotograph the reticular membrane is in focus. Note the outer hair cell in fifth row. (Magnification 1360.)

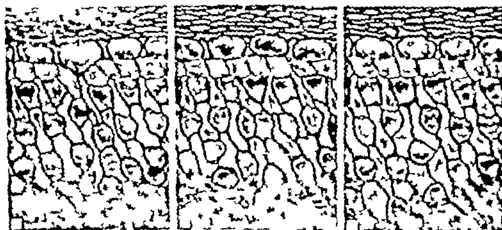


Fig. 15. Different types of irregularities occurring in the pattern of sensory cells. F... month human fetus surface preparations from the lower middle coil.
A. Irregularities of inner and outer pillar heads (... row) and neighbouring outer hair cells.
B. ... outer hair cells lying close to each other without supporting cell between them.
... Irregularly placed outer hair cells. This is one of the commonest types of irregularity. (Magnification ... and ... 455.)



Fig. 18. Org f C rti f f e-month hum fetus, surface preparation from the low basal coil showing rregul rity f cell patt rn.
A. At the lev l f the free pper surface of the rgan of Corti, one out hai cell is seen lose t the row f nner hai cell (a row). The inner pilla he d l the correspondi ng region ppe rs t be missi g ade des loped.
B. When focusi g dow t deeper l el i the pithelium, t ppears as though one inne pill cell ere mi si g (rrow) N t the pace f Nuel (NS) which is w ll developed. IP indic tes the ner pilla cell OP the o ter pilla cells, and I the nuclei f the f rst r f out ha cells. (M gn fication, A nd B 1500).

faces remain mall r an l more rounded Th free urfaces f the inner h ir cell l ow the mature hape throughout ll coil In g neral the outer h ir cell are li posed in three regular rows, alth ugh cell f rming a f urth row are seen more frequentl th n at earli r tages especiall in the upper coil (Fig 15 nd 16) A in the earli r tages irregul rities of the pattern of th out r h air cell occur but with a s m wh t in reased inci lence Supernum r ry cell between the first an l sec n l or the second an l third ro w are th most comm n rregul ties (F g l) In th l a l 10 mm it occa i nall ap- pears a th gl on pill r cell w re mi ing th u ing ut r h i cell to l l w l ow f nn h i cell (F g 18) A l iation in th lin ar c nt t l ll occ r p ra li all (Fig 19)

f t atolary

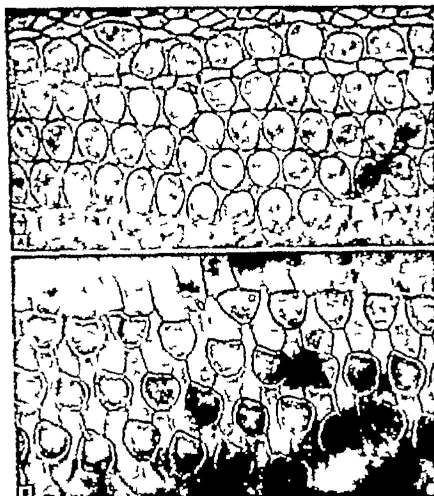


Fig 19 Surface preparation showing an interesting variety of the norm l pattern.
A Four-month human fetus, lower basal coil. Note the deviation in the lines configuration at the midfield (rrow).
B Adul squirrel monkey one coil from the basal end, showing similar arrangement of the lines configuration. (Magnification, A 890 B 1330).

At thi age intraepitheli l fluid spaces are evident in phase contrast microscopy (Fig 18 B) A spirally ru ning space is seen from the 5—10 mm region (60 —90) up to the 15—.0 mm region (about 2 0°—360) The space is located between the outer p llar cells and the first row of outer hair cells thu corresponding to the space of Nuel At the basal and apical extremities f the pace it is narrower than between these extremities. In deeper levels of the pithel um, large numbers of acuoles of varying size are observed A f w weeks later also the tunnel of Corti is partially devel ped (Fig .0)

Th piral essel i as l rge in som a eas as earlier but in other places two narrow piral vessels are seen below the inner ridge

At ix months (3. cm) the surface pattern of the organ of Corti has

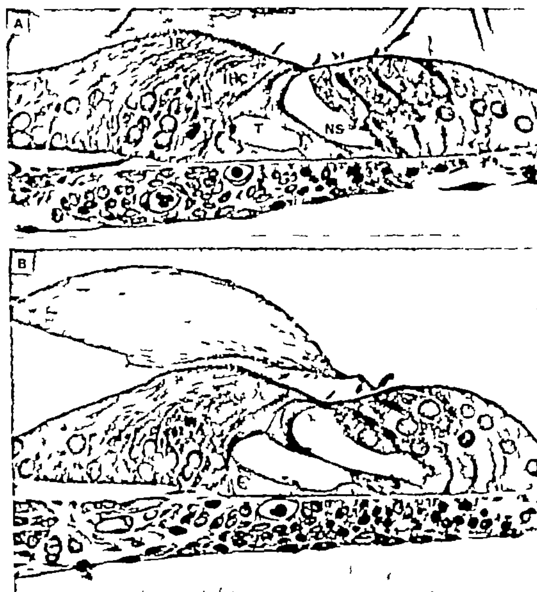


Fig. 20. Radial sections through th rga of Corti f 21-week human fetus, showi g diff rent stages f dev lopment of the fl d paces t different lev ls f the cochl

A. One coil (21 mm) from the basal end the t nel of Corti (T) is narrow the space f Nuel (NS) wide

B. H lf coil (11 mm) from the basal end the tunnel nd the space f Nuel have both widened considerably The fl id paces round the oute hai cells re lso parti lly dev l ped. N t the micro illi nd the tect ri l membrane which ha not y t become comp let ly separated from the i ner ridge (IR) The mesenchymal lay nd the basil membrane is thi k nd cont ins tw spiral essels. (Magnification A, and B 610)

changed co l rally The whole su face area ha widened increasing the distance be n tl l ır cell r ws. P rticularly striking is the el ngation of the inn ll r l l nd tl consequ ntly greater distance between tl e inne h ır cel n l w of outer h ir cell (Fig 21) Tl sensory

Art ated ry g S pl



Fig. 21. Organ of Corti of sixth-month human fetus, surface preparation from the upper basal coil (1 mm) showing the large distance between the inne hai cells (IHC) and the first row of outer hai cells compared w th that one month earlie (cf Fig. 14 and 15). Note the granules in the inner pill head One supernumerary inner hai cell is seen abov the regula row of cell The ha rs of the first row of oute hai cells (1) re in focus. (Magnification 1 40)

cells re m re w d ly dispe sed and the phalangeal p ocesses of the Deiters cells ha e hanged n ppearance to ppr ach the matu e shape Thi type of pattern is present in all co ls except f th most apical few mm where the preading t ndency is incompl te most n tal ly in so far a the nner pilla heads are con id rably shorter than elsewhere

A second triking h nge n th patt rn f sensory and supporting cells is th t rregularities ha become m re common and m re con picuous than at arli tages. Ne erth less, the regular ba ic pattern reta ned espec ally n the basal end, th i regularit es t nding t ne ease p ogressi el tow rds th apical end

Up t th i age few ign f sensory cell d gen rati n are f und Only very ra ly does a a ancy appear d noting a miss ng sensory cell. N more than 10 u h cant pl ces w re seen n all f the f tal cochl as tud ed

Th flu d paces of th rgan of Corti widen con id rabl during the xth m nth Th tunnel f Corti, which by thi tage ha become well differen i t d nd the pace f Nuel ext nd through ut the f rst two co l (0 — 0) N flu d pace are et evident in the apical co l In the basal and m ddl co l fluid paces are also evid nt am ng the outer hair cells Th

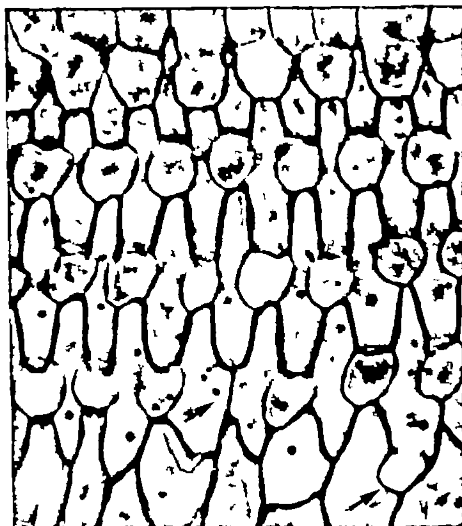


Fig 22. Organ of Corti of prem t re child (fetal age eight month) surface specimen, two coils (30 mm) from basal end, showing fou regula rows of outer ha cells and one cell i fifth row (right arrow). N te absence of one h i cell in the fourth row (left rrow) (Magnification 1480).

cytoplasm of the supporting cells show a rich vacuolization Many granules are evident in the inner pill r heads (Fig 21) though less prominently so in the most basal f e mm and in the apical coil

A ingle sp ral vessel running below the tunnel of Corti, is seen, measuring —10 μ in diamete in the fixed specimen A few essels are seen crossing the basilar membrane

At eight month the pattern of sensory and supporting cells shows the essential h ract ri ties of the adult cochlea The fluid spaces are well developed tl roughout the organ of Corti

In two ubject out f four studied t this age, a certain degeneration of outer hai ll w bserved (Fig ?_ and _3) It must be emphasized that

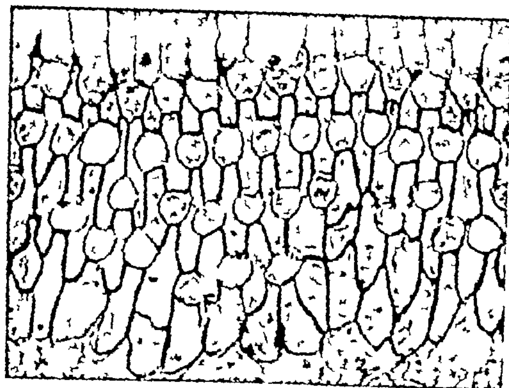
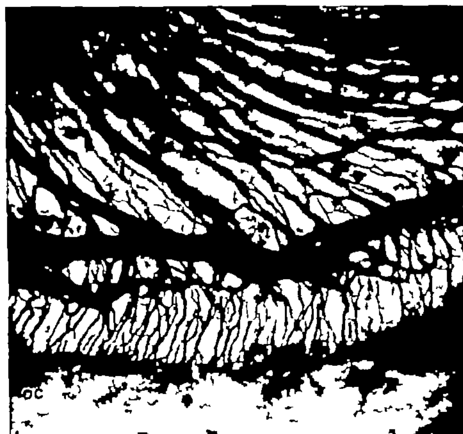


Fig 25 Orga of Corti from premature child (fetal age eight months) surface specimen from uppe middle coil (26 mm) showi g irregularit of the pattern of outer hai cells. The general rrangement of the cells in three fours rows is still present. The positions of few lost cell can be clearl seen. (Magnification 935)

th cochleas were obtained from stillborn asphyxiated premature hildren. All stages of degeneration f the hair cells were observed from the early collapse figures t the phalangeal scars The loss of sensory cells was pread d ffusel th oughout the cochlea In most places s ngl sensory cells were missing ratl e than groups of them The xtent of degenerati n was less than 5 pe cent

2 *Innervation*

In the f u month human fetus a dense n twork of nerve bundles in the future osseous p ral lam na is seen in Maillet fixed/sta ned specimens (Fig 4) This n twork is formed b rad ally and p rally runn ng nerve bundles In tl e apical co l the spiral bundles re more conspicuous than the radial From these bundles of nerves fiber run toward the rgan of Corti where they f m unm elinated bundles unde the one and outer hair cells In all coil th i n r p ral bundl and th three t four outer spiral bundles a e evident In the f e-month fetus leetr n micro-scopy reveals in the organ of Corti rather large n rve f bers containing distinct neurotubuli These f ber make contact



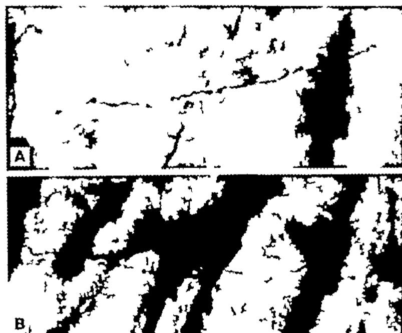
F g 24 Inner portion f spiral lamina and the rga of Corti from f ur-month huma fetus, surface specimen from the pical coil. Modified Maillet pera t la. Also are seen radial n bundles which t this lev l of the cochl tak slightly spiral course t ward the pex (t the right of the illustrated portion) The thick transverse bundles conta both radial nd spiral fibers. From these thick bundles nerv fibers run out t the rgan f Corti (OC). (Magnification 250).

with the sensory cells and form bouton-shaped nerve endings It was not possible to distinguish between afferent and efferent endings. Synaptic vesicles were sometimes observed n the nerve endings, at the outer hair cells and beneath the nner h cells.

A third group of nerv f bers is evident in the future osseous spiral lamina These are very th n fibers provided with localized swellings or beads (Fig 25) They run singly and re sparsely distributed Most of them are observed in a plan superficial t the other nerve bun lles The f bers seem to run in all d rect n and are found n the future osseous spiral l m na but not in the organ of Corti

3 *Numerical con iderat ons*

Grap I ll strat ng th sensory cell popul tions of 10 fetal cochleas are shown I f 3 Th rage number of outer l air cell wa 13 400



F g. 25 A and B Osseous piral lamina of four-month human fetus surface preparation from the middle coil, showi g ers thin unmyelinated nerv fibers with localized swellings, or bead These fibers can be seen to run bev the myelinated bundles in the spiral lamina. Modified M iller nerve stain. (M gnification 51)

ranging from 11,200 to 16 000 The inn r ha r cells a eraged 3 400 with a range between ..800 and 4 400 The numbers f inn and outer ha r cells showed a certa n correlati n w th th length of th rgan of Corti (F g 36) Th three y ungest f tuses (1 —18 weeks) of this group did not sh w notably l we values ith n the numbe f cells o in th length of the organ of Corti

The densit f sensory cells (numbe f cells pe mm length of the organ of Corti) increases from the base t w rds the apex (Figs 26—35) The outer hair cells almost double th dens ty An a rage obta ned from seven cochleas (19—24 weeks of age) showed 90 cells/mm at ne mm from basal end, and 52 cells/mm at tw mm from apex Th maximum density is found two mm from th pex, from wl i h point th numbe f cells decreases rapidly t wards th apex In th three f rst r w th numbers f out hai cells pe mm are n lose agreement w th each th howeve n the most basal and th m t ap cal f w millim ters the third ow often sh w a decrease in dens ty f cell At th age f 1 —18 weeks (3 fetuses) there are very few cells at th f urth row (Figs 6— 8) whereas at 19— 4 weeks sensory cells f rm ng f urth row occu regularly (Figs 9—35) The number of these cell ses f m n ne n the most basal few mm t an av rage of 90 cells/mm t co ls from the base There is a large variati n in the number of fourth

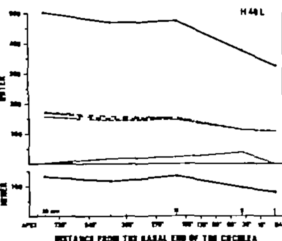


Fig 26.

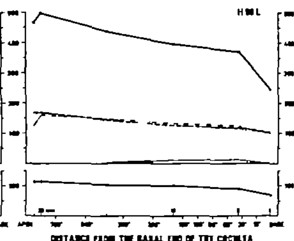


Fig 27

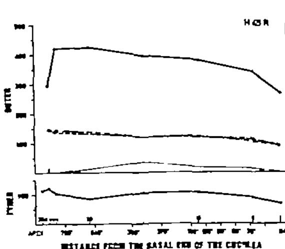


Fig 28

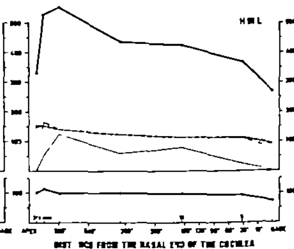


Fig 29

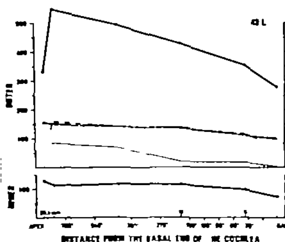


Fig 30

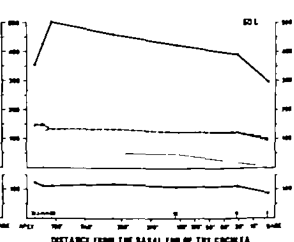


Fig 31

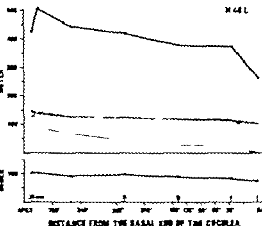


Fig. 32

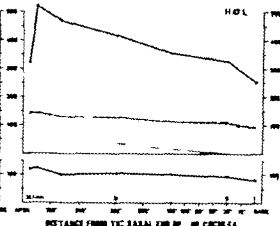


Fig. 33

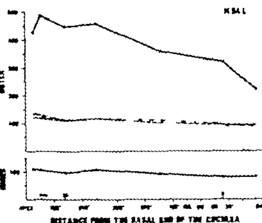


Fig. 34

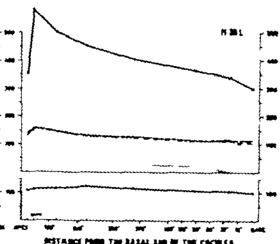


Fig. 35

Figs. 26—35. Graphs of the sensory cell populations in 10 fetal cochleas. The curves represent the density of sensory cells (number of cells per mm length) along the organ of Corti. The upper and lower continuous lines represent respectively the inner and outer hair cells, the interrupted and dotted lines representing the individual rows of outer hair cells. See page 6 for explanation of symbols.

		Number of sensory cells	
Fig. 26.	Age 13 week	inner 3630	outer 13130
Fig. 27.	Age 13	3035	12220
Fig. 28.	Age 18	3560	13350
Fig. 29.	Age 19	3170	12730
Fig. 30.	Age 19	3300	13000
Fig. 31.	Age 20	3390	13130
Fig. 32.	Age 21	3210	14120
Fig. 33.	Age 21	3350	12800
Fig. 34.	Age 21	3230	11230
Fig. 35.	Age 21	4390	16010

Note that the density of sensory cells increases towards the apex. In the three younger fetuses (13—18 weeks of age, Figs. 26—28) there occur very few cells in the fourth row of hair cells.

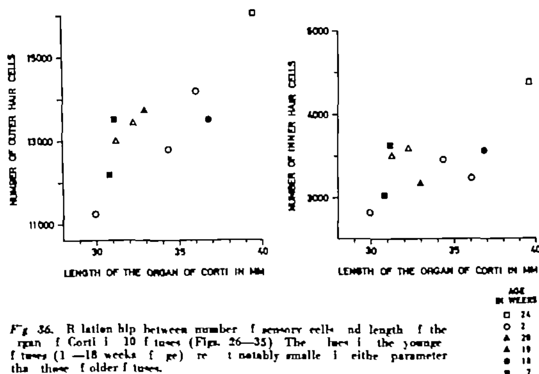


Fig 36. R latlon hip between number f sensory cells nd length f the rgan f Corti i 10 f tuses (Figs. 26—35) The lues i the younge f tuses (1 —18 weeks f ge) re t notably smalle i eithe parameter tha these f older f tuses.

row cells per mm from one cochlea to another A very few fifth row cells are found which tend to form an interrupted row They were never found to exceed 40 in a complete cochlea The fifth-row cells occur in the upper middle coil and in the apical coil In the graphs these cells are added to the fourth row counts

The number of inner hair cells pe millimeter n reases about 40 per cent from base to apex An average obta ned from seven cochleas (19—21 weeks of age) shows 80 cells/mm at a point 1 mm from the basal end and 115 cells/mm at 1 mm from the apex (Figs. 29—35)

4 *Discussion*

The morphological de elopment of the human organ of Corti is described as beginning at the basal end of the cochlea and proceeding towards the apex (Alexander 19 6 Kolmer 192 Ba t and Anson 1949 Ormerod 1960) The same sequence of de elopment is reported in most mammals studied (Retz us, 1881 Held 1909 Van der Stricht, 1919 1920 W da 19*3 Held 1926 Weibel 19 ; Änggård 1965) however as indicated from recordings of cochlea m rop h nies and act n potent al from the eighth nerve the mammalia l l beg t respond earl er t sound of medium high fre-

quencies than to low r to high frequencies (McCrady Wever and Bray 193 Larsell McCrady and Larsell, 1944 Anggård, 196 M kaelian and Ruben 196ɔ Crowley and H pp-R ymond, 1966) In accord nce w th the usual concept of frequency localizat on this should mean that cochlear ma *turation occurs earlier at a certain distance from the basal end than at the* basal and apical extremit es M rphological development in agreement with this functional interpretat on has been described in th mouse (Lorente de No 1933 a M ka lian and Ruben 1965) and in the opossum (Larsell, McCrady and Zimmermann, 1935 Larsell McCrady and Larsell, 1944) The results of the present investigat on indicate that d fferentiat on in the human cochlea also begins at some distance f om the basal end and progresses in both directions. This was observed in *respect* of both sensory cells and fluid spaces

As menti ned above all electrophys ol gical experiments to dat have suggested *a maturation in general agreement amongst different species, where* as histological invest gations ha e fa led to demonstrate a imilar agreement It would seem most probable that tru tural development would proceed in a sequence parall l to that of functional maturation On the basis of comparati e anatomy it also seems unl kely that th similar de elopment n the opossum in the mouse and in the human would be diff rent from that in oth r mammals (bat guinea pig, rat, rabb t, cat) The apparent disagree*ments are probably* due to technical problems In conventi nal serial sec ti ning f the cochlea, th l wer ba al coil of the organ of Corti i difficult t e aluate A second *f* ct r *is that* maturat on *is rapid, especially in the* smaller mammals In thei condensed time-scales of maturat on, it would be more d ff cult to study the cochlea at the exa t t me whi h would reveal the sequential deta ls In th relativ l lower time scale of the human organ of Corti, f instance th basal lag in devel pment f the sensory cells was l arly observed only t th age of three m nths. It is possible that an elect on m c oscopi tudy of development might solv the problem by the use of m re subtl rit ria

In accordance w th prev us tudies (Held, 1909 Cajal, 1919 Wada 19 3 *Lorent de No 1926 K lme 19 and others) it was f und n the present* in est gation, th t th differentiati n of the inner hai cells precedes that of the ut

An ff rt wa made in tl present study to est mate the full complement f sensory cells n the fetal organ of Cort This i poss ble nly if the f tuses re tudi d t uch a tage f dev l pment that tl eir cochlea sensory cells are ll n place Kolmer (19) stated that n further cell division occurs ft the tim tl t th cells ha differentiated into l ght, supporting cells l d rk sensory cell In th oungest fetus (three m nths of age) in th present in estigat on it wa mpossible under the l ght microscope to diffe t t se sory cell from supporting cells in the apical 5—10 mm (and outer l cell n th most basal few mm) In th upper portion f the regi n l sensory cell could be identif ed, these were more densely packed than tl *corresponding regi n of a m re* mature cochlea howeve when th

number of identifiable sensory cells was calculated it did not reach the number obtained two months later This indicates that additional sensory cells either were formed by cell division or were already developed although not yet identifiable under the phase contrast microscope Ruben (1967) showed that the apical sensory cells of the mouse ceased to divide earlier than the basal cells (see page 12) Thus, proliferation of cells in the apical coil does not seem to offer a likely explanation Further Ruben (1967) suggested that the growth area in the organ of Corti might be at the basal end Thus growth by cell division would cause the apical part, its cell division already completed to be pushed further apicalward However the cessation of mitosis was observed at a very early stage; at an age in fact, that probably corresponds to less than three months in the human fetus. The regular arrangement of the sensory cells in the basal coil with no signs of cell division, also argues against this mechanism at the age of three months hence it seems probable that the apical (and most basal) cells of the organ of Corti have already differentiated into sensory and supporting cells at the age of three months even though this cannot be confirmed by light microscopy

There is one finding which is not obviously or entirely in accordance with the foregoing discussion In the younger fetuses studied, (age less than 19 weeks) there are very few cells which occur as fourth or fifth row cells A possible explanation of this might be that the fourth row cells although essentially differentiated at that age have not yet taken on the fully developed appearance of mature cells as have their neighbors in the first three rows. Another factor must also be considered, namely that simultaneously with the appearance of the fourth row cells, irregularitis become more common in the first three rows The occurence of divisions during the fifth month would explain the appearance of fourth row fifth row and supernumerary cells in the first three rows, as well as the irregularity of pattern of the outer hair cells as a whole The higher incidence of supernumerary fourth and fifth row cells, and the general irregularity of pattern in the apical coil, would then bespeak a greater frequency of mitoses during the fifth month in that region These findings fail to coincide w th those of Ruben (1967) that in the mouse mitotic activity ceases to occur earlier at the apex than at the base of the cochlea however it is to be noted that as in most other sub-primate mammals studied the mature pattern of sensory cells in the mouse is very regular fourth row cells appearing only occasionally (Engstrom Ades, and Anderson 1966) Thus it appears that mitosis in the apical region of those animal is essentially complete by the stage corresponding to the beginning of the fifth fetal month of the human A relatively longer persistence of mitos among the outer hair cells of the human organ of Corti might account f the more irregular and distinctly different pattern of cells between man and other mammals which have been studied

Irregularities in the organ of Corti in some monkeys similar to those of man have been observed Johnsson and Hawkins (1967) described in on rhesus monkey and in one pig tail monkey a pattern in the reticular mem

brane which was virtually identical with that of man In the squirrel mon key Ades, Bredberg, and Engstrom (unpubl data) and Bredberg (1968) found a s milar but less pron unced irregularity especially in the ap cal coil As compared with the human cochlea *very few sensory cells were observed* in the fourth row position.

Some types of irregularities in the human organ of Corti occurred only infrequently giv ng rise t the quest on as to whether these were to be regarded as normal variants or as malformations One uch example is to be f und in the linear de ation of the pattern of outer ha r cells (Fig 19) which was first observed in one fetus As the study continued, and as nterest be came focused on the typing of various irregularities, the same pattern was observed frequently in both fetuses and adults A s m lar deviat on was also seen in ne squirrel m nkey Such observations tend to take this particula *irregularity out f the category of malformation* however in one 18-week fetus, a true malf rmation of pattern was f und both labyrinths how ng the Mondini Alexander type of abn rmality (Fig 37) (cf Ormerod 1960) In one of the ears the organ of Corti was 13 mm l ng Sensory cells could be identi fied throughout this length, most of them n a normal stage of development, the inn hair cells forming the customary s ngle row the uter ha r cells also sh wing a generally normal arrangement The principal dev at on was found in the ap cal co l wh re the outer hai cells formed f ur five or six row A few outer hair cells in the basal co l were abnormal in the sense that the hair bundles were malf rmed, some hav ng tereo-cil a distributed diffusely ov r the free surfaces others h w ng an inversion f the normal W pattern of hair distributi n (F g 3)

The ixth lunar month sees a considerable alterati n in the pattern of sen sory and supporting cells n the organ of Cort It is m rked by the expansi n associated with th dev lopm nt of the fluid spaces wh ch cann t be related to a corresponding increase in numbers of cells The total number of sensory cells aries cons de ably f om one cochl a to another and the number of cochlea studied wa mall in tatistical t rms nd for this reason it wa mpossible to ident fy a small in rease in the numbe of cells with increasing ag E en during th fifth month, when most of the f urth row cell normally appeared it was not possible to d monstrat by total cell counts significant ncrease n numbers Onl by *tatist cal analy* s of counts in a much *larger* populati n of cochl a could th significance f such small hanges be assessed The present tudy did n t fford su h numbers and it can only be said th t there was no maj increase in the t tal number of sensory cells during tl f fth and xth m nths.

Th hum n cochlea is described as morphol gi lly mature at th fetal age f x m nth (Bast and Anson 1949 Ormerod 1960) In apparent con t dict n t this t was found, in the present material that the fluid spaces w re *n t compl tely dev l ped in the* pical coil at this age however only n such f tu w a a lable for study Ina mu h as det rminati n of f tal age t be mad with absolut accuracy and the devel pment is rapid and



F g. 3" Mondini-Al ade type of malf rmation f the inner i 6 e-month human fetus.

A. The cochlea after remo l of the otic capsul by microdissection. The piral lamina (SL) nd associated rga of Corti b bee exposed The region of the round window (RW) is seen on the right of the photograph, the apex of the cochlea on the left the t tal length f the rgan f Corti being 13 mm The cochl consist only of half a basal coil and twisted piral portion. N te the bundles of ner fibers in the spiral lamina. The missing p rt f the rga of Corti and piral lamina (indicated by the rea between the rrow), was remo ed f the preparation f the surface specimens shown in B, C, and D below (M ndic tes the modiolus)

B. M lformation of one outer hai cell (rrow). The hairs re distributed diffusely ov r th surface f the unus ally la ge cell.

(and D. M lformation of one hai bundle shown g ra real of the haracteristic W (rm two (focused on cell surface D on hai bundle Note that the k leilium occupies it norm l pos tion. (Magnification, A ×11; B C, and D 1310)

individually variable our findings are necessarily less than conclus e n thi respect

Previous stud es on the development of the innervation n the organ of Corti have generally ndicated that sensory cells in the basal co l receive n rve fibers earlier than those in the ap cal co l (Cajal, 1919 Lorente de No 19*6 Tello 1931) The fetuses used in the present in estigati n were of such advanced age (4 to 5 months) that they offered little pertinent evidence on this point.

According to Maillet (1963) the osmium tetroxide zinc iod de technique reveals several types of structure containing l poproteins, such as peripheral nerve endings, preganglionic and postganglionic fibe s of the autonom system, and m tor end plates In th organ of Corti the n rve endings heavily stained by this technique were considered to be predominantly efferent (Engström Ades and Andersson, 1966) In the present study both radial and sp ral fibers n the osseous spiral lamina took up the stain. In add tion th rd group of nerve fibers, provided w th localized swellings or bead was re aled. It might be possible that these fibers are autonomic In the rgan of Corti sp ral bundles (inner and out r) were heavily tained It wa not pos ble howeve t evaluate to what extent n rve end ngs took up the sta n

On the basis f m rphological criteria t has been ssumed that respons e ness to acoustic stimulati n would appea at the fetal age of s x months (Bast and Anson, 1919 Ormerod 1960 Wedenberg, 1965) howev r in animal experiments, t has been shown that the cochlea may begin to rea t electrophysiologically before it is enterely mature m rphol gi ally Thus the flu d spaces w re found not t be fully developed bef re response to sound stimulat on could be el c ted (Larsell McCrady and Larsell, 1949 Mikaelian and Ruben, 1965) Kikuchi and Hild ng (1965) found the afferent nerves and n rve end ngs of the mouse to be present several day bef re fluid paces were form d The appearance of the efferent nerve end ngs on the other hand was delayed until after the tim wh n it has been possible to elicit microph nics and action potentials from the eighth nerve (Mikaelian and Ruben 1965) The animal experim nts thus suggest the possib lity that th human f tal cochlea may begin to react t sound st mulation bef re th age f s x m nths.

The quest on of funct onal maturation of the ochlea has mportant clin cal impl cations relating to the criteria fo the peut aborti n Several countries now permit such operations where the is compelling reason to believe that the m the might produce a hild w th som serious dev lopmental defect Th rapeuti abortion can be carried out n Swed n if d ne in or bef re th 4th week of p egnancy The early d agnosis f dysfunction in the organ of Corti and th mplicit probabil ty f cochl damage might contribute igni ficantly to the decisi n f r and ju tification of a th rapeutic aborti n; for exampl in ses f matern l rubella Wedenberg (1965) reported that reac t ons to sound may be recorded f m the f tus during the 6th week of preg nancy using techniqu de l ped by Johansson Wedenberg and Westin

(1964) If the cochlea is capable of functioning earlier than the 24th week, it might be possible to develop hearing tests which could be made at such time.

The most common causes of perinatal hearing impairment are prematurity neonatal asphyxia birth trauma and icterus neonatorum (Wedenberg 1965) These may occur singly or in various combinations with each other Likewise according to several reports of audiological investigation of such cases, the location of the underlying damage may be in the cochlea the central auditory pathways, or both Only a few histopathological studies are available Hall (1964) described a reduction of ganglion cells in the cochlear nuclei resulting from neonatal asphyxia while changes in the cochleas were interpreted as artefacts Buch (1966) described a compression of the organ of Corti in cases of neonatal asphyxia though he was unable to rule out artefacts, and was, therefore unable to establish a causal interrelationship

In the present study a moderate diffuse loss of outer hair cells was found in two out of four stillborn premature children none of whom showed signs of RH or ABO incompatibility The damaged sensory cells showed all stages of degeneration from collapse figures to phalangeal scars. The latter type would hardly have been expected if the damage had occurred during the asphyxia In adult animals exposed to noise or ototoxic drugs the formation of a complete phalangeal scar takes at least two weeks This being so it seems unlikely that all degeneration of sensory cells can be due to the acute asphyxia unless the resolution occurs much more rapidly in premature children than in adult animals If the degeneration of sensory cells in both fetal and adult cochleas occurs at a similar rate one possibility might be that only the acute signs of degeneration were attributable to the asphyxia while the phalangeal scars had some othe etiology The possible assumption that degeneration of sensory cells may be a normal occurrence during fetal life is not supportable by ou observation Two premature and two full term new born children were found to show no such degeneration at all It seems im probable that two independent etiological factors should con erage in two fetuses each factor producing its separate pathological effect A more rea sonable explan tion of the loss of cells in the two stillborn premature infants would be a prolonged fetal affliction due to toxaemia of pregnancy diabetes, infection, or the like In such nstances the prematurity and the asphyxiation m ght be secondary t these maternal condit ons. The degenerat on apparently of l ng standing might then be ttributable to a toxic effect on the fetus long bef re birt the final asphyxia contributing the addition l acute damage In any case even though the number of premature children was lim ted the fact that half of them l owed no ig of degeneration would indicate that pre maturity and sphyxiati n l t ecessarily *per se* produce damage to the organ of C rti

B STRUCTURE OF THE POSTNATAL ORGAN OF CORTI

1 Population of sensory cell

a. The full complement

At the outset of this invest gat on the plan was t use the ears of the younger age group as normal material, that is a a baseline for the def ni-tive study of ageing and pathological alteration in the cochleas of the olde subjects This plan appeared to be a reasonable one in the light of man statements n the literature to the effect that cochlear sensory cells tend t be all present and inta t in young nd viduals with normal hearing. There are statements to be found which go even farther to nd cat that even in eld rly subjects with severe impairment of hearing, inta t sensory cell populations may be seen At least the last statement could howe er n t be verified by the present study The cochlea of the newborn infant showed a compl tely intact population of cochlear ha r cells but this w the latest appearance of such a phen men n. Even th cochlea of olde h ldren and young adults, in cluded in this stud displayed appreciable degrees of cell loss As a conse quence t became necessary to find a basis f d finition of the completely intact populati n of cochlea sensory cells. Inasmuch as the newborn was the oldest individual showing no evidence of cell loss, the later f tal material was used for this purpose Acco d ngl th full complement is def ned a that wh ch i seen n the fetal cochleas n whi h the development f sensory cells is complete as determined by hist logical criteria

The material a a lable fo thi purpose consists of seven cochleas a number which is too small to perm t a statistical anal is that will gi e additi nal in formati n In the graphs of cellula populations of the postnatal cochleas the range of variation in dens ty of the fetal nn r and oute ha r cells is repro-duced as the hatched rea (F gs 48—88) thu serv ng as approximate n-dicat rs of the intact population of sensory cells

b. Degenerat n of sensory cell

Although the patt rn of sensory cell l s v ries ons derabl in diff rent cochleas it is evident that the extent of d generat n becomes more pro-nounced with ncrea ing age Certa n general princ ples with respect t xtent and locati n of this degenerat on eme ge from th 41 cochlea whi h were stud ed in a d ta l d sy tematic way The sensory cell los in its relati n t ageing w ll be discussed first foll wed by th t related to degenerat on from other causes It must be empha ized that the descript n f the degenerati n pattern associated w th ageing ref rs nl to th e present materi l, and, alth ugh thi i h sen rand mly with respect to hearing los and n se ex posure it may at t mes how deviati n f om what is generally regarded a usual fo gi en age Th w ll be discussed further in late paragraphs

Degene at on of nte h ell with ageing Th rat of reduction of hair cells diff rs between nne nd out hai cell the latt d generating at

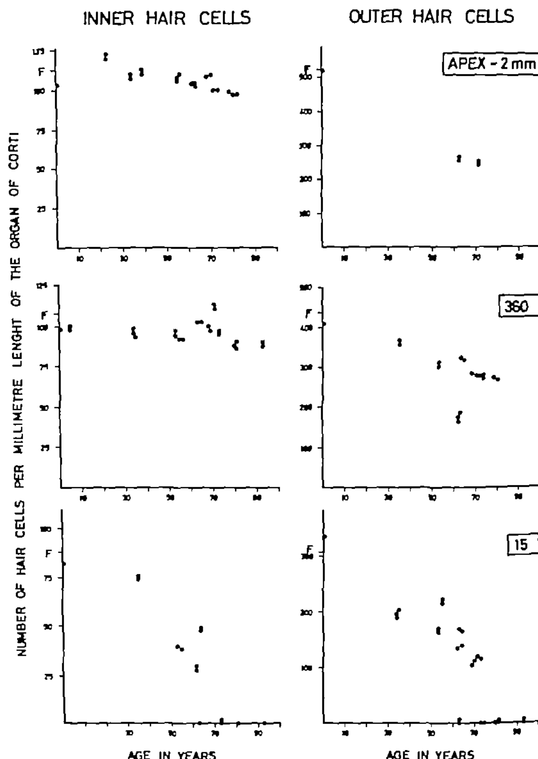


Fig. 34. Relationship [illegible] different levels [illegible] the cochlea between sensory cell density and age. F indicates the full complement of cells (average obtained from [illegible] fetuses) at each level. At the basal end (1 [illegible] 3 mm) both inner and outer hair cells show considerably lower density with increasing age. [illegible] (360 [illegible] 21 mm) from the basal end only the outer hair cells are reduced with age [illegible] half the amount found at the basal end). In the apical coil (Apex — 2 mm) the reduction [illegible] density of outer hair cells [illegible] ageing is considerable whereas that of the inner hair cells [illegible] minimal.

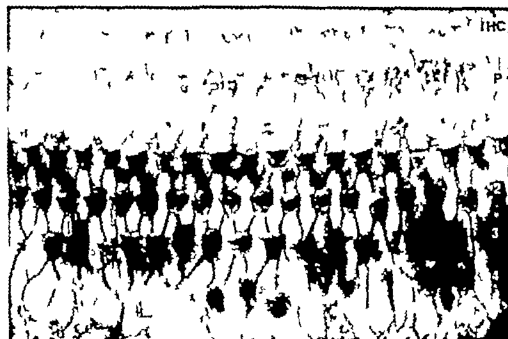


Fig 39 Organ f Corti of an 18 yea ld man, surface specimen from the ppe basal coil (1 mm) showing the ppearance of the ad lt pattern. The h rs of the inner hair cell (IHC) re in focus The oute hai cell (1 2, 3, 4) from hree four T oute hair cells re missing f om the th rd row P indicates he nner pilla head (Magnification 830)

almost double the rate of th former (Figs 93 and 94) Cell loss begin and becomes not ceable bef re the th d decade and seems t be accel rated in lder age groups.

The degeneration of out r hair cells occurs th ughout th cochl th ugh not at th same rat n all regi ns Th lowest rate of degenerati n occurs in the region 2 0 —510 (1 — 6 mm) from the b sal nd where at the age f 50 th loss of cells is about 5 per cent The at ncreases from this region t wards both the basal and the pical ends almost doubl ng f m the cent t either end Th loss of cells sligl tly more pronounced at the b sal end than at the apex (Fig 38)

The distribution f cell loss among th different row was calculated by measuring th erage dens ty of cells t f e lev l (60 135 0 360 and 540) of all 41 cochleas Since th cells f rm ng the f urth r w are distributed differently from the cells n th othe row it was not possible t compare th damage in this row w th that of the oth row At 0 360 and 540 the th d w sho greate cell loss th n the other rows Th maximum d ff rence wa at 360 wher the th d row h wed about 15 per cent fewer cells than th first and second row Th difference was tatistically ignificant a tested by the h squ r method on ranked data

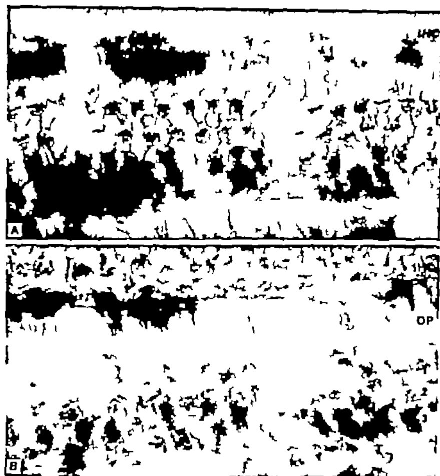
Degeneration of inner hair cells with ageing The highest rate of loss of inner hair cells is found in the basal coil (Fig 38) Measured at the lowest extremity (15° or 3 mm from the basal end of the organ of Corti) the rate is nearly as high as that of the corresponding outer hair cells From that point apicalward the loss diminishes to a minimum at 360° from base achieving there a level of slight loss which then remains uniform throughout the rest of the distance to the apex The rate of degeneration in the lower half of the basal coil (0°—180°) appears to accelerate with increasing age after 50 (Fig 38)

A comparison between the pattern of degeneration of inner and outer hair cells thus reveals distinct differences *The inner hair cells show a basal degeneration only whereas the outer hair cells show an overall degeneration which is accentuated at the basal and apical ends* however there is considerable individual variation from this general pattern the basal loss predominating in some cochleas, the apical in others Still others show a more diffuse loss of varying degree

Other forms of sensory cell degeneration In about one third of the 41 cochleas studied a completely different kind of sensory cell loss is found This consists of a circumscribed loss which is related to neither the basal nor the apical degenerations described above This kind of degeneration shows a considerable variation in extent and location The area of maximum damage is usually found in the region from 7 mm (60) to 21 mm (360°) from the base Two major variants may be identified One of these affects the outer hair cell population with partial loss of cells over a wide area (5—10 mm) the third row often showing considerably more damage than the first two The second consists of a serere lo s of both inner and outer hair cells over



Fig 40 Cochleogram from cochlea H 101 R (Fig. 68) from the region between 90 and 135 (9—11 mm) Note that the missing hair cells are located in groups. This subject was during life exposed to noise and the audiogram revealed a dip in the frequencies 1000 6000 cps. ( ) represent intact sensory cells and (●) missing cells.



F g. 41 Orga of Corti of 18 ea ld m n, surface preparation from the lower basal coil (9 mm) howing degenerat of te ha cell and pilla cells.
A When the microscope is focussed to the reticula membrane, t groups f oute hai cells re seen t b degenerated. The region of the inner hai cell (IHC) i not in focus but, as seen in B below no cell were lost.
B When focussed more deepl in the epithelium, the outer pilla cell (OP) re seen t be missing in those rea which correspond to the lost groups of outer ha cells. No I ner hai cells re missing as indicated by thei intact nuclei (IHC) 1, 2, and 3 indicat the rows of outer hai cells. (M gnification, A nd B 685)

a shorte distance (less than 5 mm) In few cochleas several such circumscribed areas my be seen In addition t these ob ious les ons of the popul t on of sensory cells, there re tl rs consisting of ery narrow reas (m t of th m less th n 0.5 mm) sl wing lo of eithe nne or ute l air cells.

2 *Suppo ting element nd tect rial membra*

Th damage seen n tl e organ of Corti by light micro-copy ranged from diffuse, light sensory cell los t m re ten i e lesi s Th sensory cell



Fig. 42 Outer hair cell region of the organ of Corti of 73 year old man (cochlea II 109 R. Fig. 53) Surface preparation from the upper basal coil (14 mm) showing loss of about one third of the outer hair cells. The degeneration is ... and in the second (2) and the third (3) rows. (Magnification 1250)

losses, are roughly paralleled by a progression of changes in the non-sensory elements of the organ of Corti

In the cochlea displaying the minimal diffuse type of damage with occasional single hair cells missing here and there (Fig 39) there is generally no noticeable change in the supporting elements The next identifiable stage shows a more pronounced loss of sensory cells, involving groups of adjacent cells in one two or all three rows of outer hair cells (Fig 40) In such instances if the damage involves the first row predominantly or in combination with the other two a few outer pillar cells may also be lost in the same area (Fig 41) If the damage extends to and involves the inner hair cells also the change in supporting elements is usually more pronounced often including inner as well as outer pillar cells It must be noted however that pillar cell damage does not invariably occur even with extensive hair cell degeneration (Figs 42 43 44) On the other hand a sensory cell lesions of similar extent may be associated with collapse of the Hensen cells and all other supporting elements outside the tunnel of Corti in which case the collapsed portion often replaced by a single layer of flat polygonal cells. Such lesion may ... row of corresponding inner hair cell

Still another ... hown a circumscribed region where both inner and outer hair cell ... this region showing an organ of Corti completely collapsed and ... while the Hensen cell are ... an appear-



Fig 43 Organ f Corti of 73 yea ll m (cochle ll 109 R Fig. 53) surface preparation half coil (11 mm) from the basal nd showi g extensi d generation of both inner (IHC) nd outer (1 2, 3) h i cells. Three i ne nd eight i h l ll re present (Magnification 1250)

ance of near-normality Such lesion t n l to be limite l t a lin ar di tanc of less than 1 mm a d when l th inn r an l ut r hair cell are lost ov r a dista ce of more th n 1 mm th wh l e rre pondi g regi f th org n of Cort is usually alm t c ll pse l Th i type i often seen i the m t ba al f w mill meter of th cochlea F n with c mpl t collapse th patt rn of ph l ngeal scars can till be i l ntified i th remna t of reticular m mbran still present On th oth er hand similar ir umscribe l lesi n elsewh re than n the basal nd t nd t bel m what diff rently in that th organ of Cort i l rg ly replaced by a thin lay r f polyg n l cells, no reticular mem bran remai s, n l h n the haract ri tic p l al g al scar patt rn cannot be seen

Th tect rial m mbran ov red the h ir cell region of the organ f Corti i all of the cochlea tu li d (Fig f) In the course f dissecti n in some pecimen it w f un l that the hair tuft of the out r hair c ll adhered t the membr ne The ites of uch cont t between sensory hairs and tect rial m mbrane were e ily id nt f d upo remo l of th m mbra by virtue of the imprint l ft on it und r s l The hair bundl f the th ree fir t row f outer se sory cell are nnect d with th o t part of th meml r n th

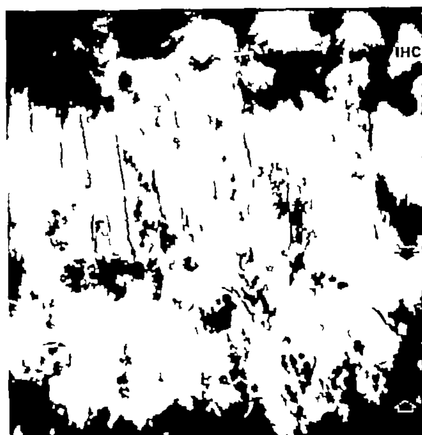


Fig 44 Orga f Corti f 93 yea ld ma (cochlea H 90 L, Fig. 52), surf ce prepara tion from the apic l coil (2 mm from pex) ho ing t tal lo f out ha r cells. The inne hai cell re till present nd thei hairs re i focus (IHC) The uter hai cell re (between rrows) sh w irregula frame rk f supporti g cells. (Magnification × 1.65)

third row being just at the border of the H ndfasernetz (Figs 46 and 4) The W-shape of the arrangement of hairs is clearly visible The fourth and fifth rows make contact with the H ndfasernetz No similar sign of contact between hairs of the inner hair cells and the tectorial membrane was observed

In areas where the organ of Corti was completely degenerated the tectorial membrane was always observed n a fairly normal position i.e., it wa never depressed towards the ba ilar membrane nor folded toward the Reissner membrane

3 *Nerves in the osseous spi al lamina*

In the late fetal and in the premature material the radial nerv fibers appear evenly distributed in dense bundles throughout the cochlea except that in the most l asal millim t r tl y re more sparse The spirall running bundles can be discerned but th course and is are diff cult t evaluate a the more densely distril uted ra l l fibers ten l to ol scure tl em



Fig. 45. The most apical quarter of coil of the osseous spiral lamina with associated organ of Corti and basilar membrane. The tectorial membrane (MT) covers the hair cell area of the organ of Corti. Between this membrane and the area of the Hensen cells there is a narrow region which contains outer hair cells of the fourth and fifth row and is covered by the Randfasernetz. The basilar membrane is seen outside of the Hensen cell area. H indicates the helicotrema. (Magnification 40).

It must be emphasized that in the present study the evaluation of nerve supply was based on observation of the nerve bundles in the osseous spiral lamina as seen in whole preparations or in surface specimens. It is not possible, therefore, to offer any precise quantitative information on innervation density. Nevertheless, the overall view of the innervation gives a fairly accurate estimate of density and regional differences are especially well displayed.

In the newborn the radial bundles in the most basal few millimeters of the osseous spiral lamina show a lower density toward the basal end, the only deviation from the otherwise uniform distribution. With increasing age there is a rarefaction added which becomes more pronounced and extends further apicalward. In older age groups this degeneration is often complete in the most basal 2—3 mm. This phenomenon extends above this limit only in very few cochleas (e.g. Fig. 53). With ageing, a slight rarefaction extends throughout the basal coils and beyond the age of 60 may spread even farther apicalward (Figs. 49—53).

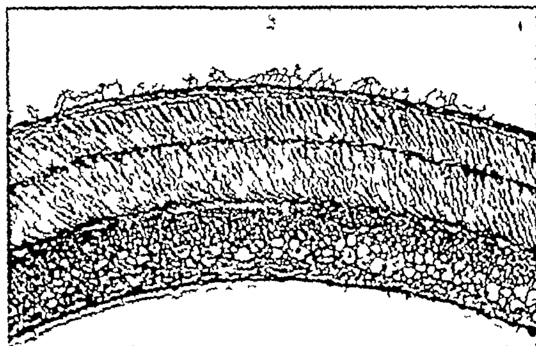


Fig. 46. Tect rial membrane removed from the pper middle coil as seen from its ader surface. From bo downw rds re seen the Filamentos Randfasernets the rrow stripe of Hensen (SH) and the relativ l ro d rea l contact with the piral limbus (L) (M gnification 115)



F g 4 P ripheral m f the tect rial membrane one nd half coil from the basal end show ng the impri (H1 H H3) of the h bundles of the f rst three ro of out ha cell The f h nd f f th row m k the ontact w th the Randfasernets (R) lthough no such of   be seen in this particula spec men. (M gnification 0)

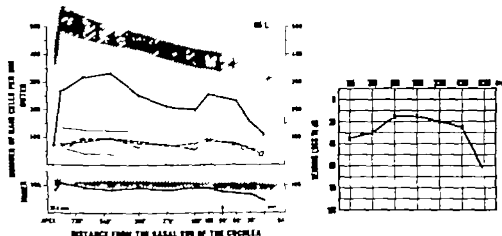


Fig 48 Cochlea from m ged 56 years, basal co l. No history of noise exposure or ea disease Autopsy diagnosis hypernephrom cardioatherosclerosis, nd myor cdi l inf rction. Treated for 2 month with the cytot ic gent cyclophosphamid (Sendoxan) to tot l dosage of 2 g.

The cochlea show an even distribution of nerv bundles throughout the osseous piral lamina. Only the basal coil f the cochlea is shown in this photograph. The rrow indicates the poi t one coil from he basal nd, here which the middle nd piral coil ha been removed.

The graph shows an overall reduct on of oute hai cells which is accentuated at base and apex. I addi ion there is circumscribed reduction the density f outer hai cell i the region 180 —360 The inner ha cells reduction in den ity t the base T tal number of hair cell outer 20 inner 2670 Length of the org of Corti, 31.2 mm. See page 6 f an explanat on of he ymbol used in the present graph nd udiogram, and in those whic h f llow



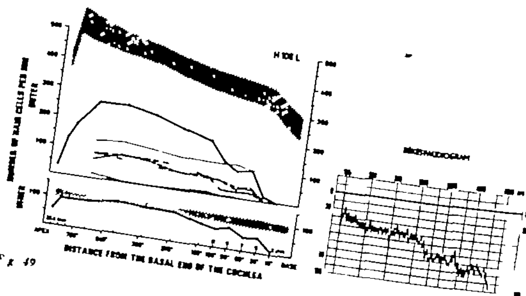
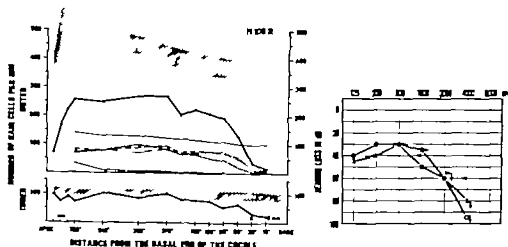


Fig. 49 and 50 Cochl cell populat on graphs, pure tone udiogram nd Békésy ud o-gram from man ged 60 ea s. E posed d ri g lif t oise from rock-blaster and mara-dam-breaker. Aut ps d gnosi bronchial carcinoma, cardiothe osel rosis, nd myocardial infarction. Tre ted w th 1–9 g. Send xa one bef re death (bef re udiogram w re btained)

The left cochle (F g 49) h pronounced degene t on of ners f be t the basal end (0—60 0 mm) nd sl ght ov rall degeneration extendi g throughout the cochlea. In the pical en l the degen tio m re pronounced i circumscribed region. The right cochlea howe l sim l h



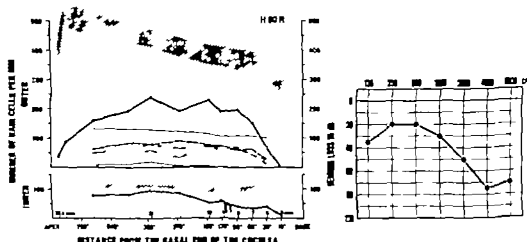
F g. 50

The sensory cell populations show simila ppe rance in both ears (left Fig. 49; right ea Fig. 50) Out hai cell extensive degeneration throughout ll coils. The basal los l more pronounced than that at the pex. Inner hair cells basal degeneration extending t 2"0 Number f hai cells left ear ter 5100 inner 2610 right ea outer 6250; inner 2150. Length f the rga f Corti left ea 31.8 mm; right ar not measured, so that the numbers of sensory cells i thi ea correspond to the rage length of 34.0 mm.
Pure tone udiogram f both ears re shown i Fig. 50 Békésy audiogram f the right ear was similar to that f the left ea shown i Fig. 49 Speech discrimination score 56 ; speech threshold, 50 dB i both ears.

Several cochl as howed f rly large, ircumscribed reas f compl t loss of nerves in the basal coil. The d tribution of these les ns is somet mes patchy (Figs. 55 56 nd 89) In nly one pai of cochle (Fig 59 nd 60) is such a degeneration seen further apicalw rd These ears show a patchy l ss f nerv fibers throughout the ent re cochle In some reas the loss is compl te whereas n others the dege erati n is parti l In the apical coils, circumscribed area of rarefacti n f nerve supply in the osseous spiral lamin was observed in som cochleas.

In addition to th xtens e les on menti ed abo e, th re occur in about on f urth of the coel lear m ll ircumscribed areas f neural raref cti n o compl te l ss of nerv fibers Th type f dege erat n is often vi ibl o ly in the region lo e t th h be ula perf rat and the lesi ns re seld m m re than 0.5 mm n width Tl ey m y be multiple ingl

As mentioned previously th pirally ru ning fibers in the osseou spiral l min w re difficult to t ly i d t ll in cochlea with largely intact radi l l nerv n From t dies n cochl l wing rying extent of degen ra ti n n the radial nerve fibers (Figs. 49 1—53 5—60) the d stribution of th p ral fibers seem to off eon d ral le ri ty f t ly The bundles ry n thick ess d numbe In tl most ba al quart of co l (0—90) the p ral bu dles re few l thi In cochl N H 109 R (Fig 3) where th degen rati of radi l fibers i ten l tl p i l fibers also t nd t



F g. 51 R ght cochle from ma ged 93 years. N hist ry of noise posure disease. A t psy di gnosis ncer f p ostat with metastases.

The cochle shows complet loss of nerv f bers i the basal 3 mm. The density of nerves gradually inc eases p t 270 (1 mm) nd light ov rall rarefaction persist t this position and t ward the pex. In the regio between 90 nd 135 (9—11 mm) there re tw rea of lmost complet loss f nerves, and i the region bet een 135 nd 180 (11—13 mm) few rea f minor rarefaction seen

Oute ha cell popul tion tend loss, more pronounced t the pe than t the base. Inne hai cell basal degeneration extending t 270 (21 mm) I tw reas the region between 90 d 13 ach bout 0.3 mm wid nd co responding t an rea f nerv fiber loss, the ame h ll re t l ge extent degenerated. Number f hai cell oute 5700 inne l t. Length of the rgan of C rt 31.8 mm

Note that although l l d generat on of oute hai cell more pronounced tha that t the base there l light hearing loss f l w tones.

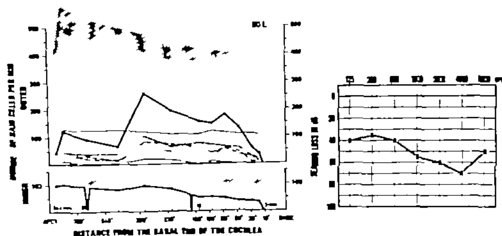


Fig. 52. Left cochle [illegible] with graph [illegible] f sensory cell population and audiogram, from the same subject [illegible] Fig. 51. The nerv [illegible] bundles in the osseous spiral lamina show [illegible] overall degenera [illegible] on [illegible] mil [illegible] t tha [illegible] f he right ea [illegible] I [illegible] tw [illegible] narrow regions, 14 mm and 28 mm respec [illegible] iv [illegible] l [illegible] f om the basal end, the nerv [illegible] bundles [illegible] re more grossly rarefied.

Ou [illegible] hai [illegible] cells [illegible] degeneration, leaving i [illegible] the apical 10 mm only about [illegible]0 [illegible] art [illegible] cells. Inn [illegible] ha [illegible] cells [illegible] basal degeneration extending up to [illegible]0 [illegible] In two areas, 0.1 mm [illegible] nd 0.8 mm [illegible] idth, there is [illegible] lmost [illegible] complete loss of cell [illegible] corresponding to the [illegible] f [illegible] f [illegible] her loss. Number of ha [illegible] cells: oute [illegible] 4250; inner [illegible]. Length of the [illegible] rgan of C [illegible] 33 [illegible] mm.

Th [illegible] show [illegible] more pronounced [illegible] piral loss of outer hair cell [illegible] and [illegible] more severe he [illegible] ring [illegible] mpairment f [illegible] low tones tha [illegible] the right ea [illegible]

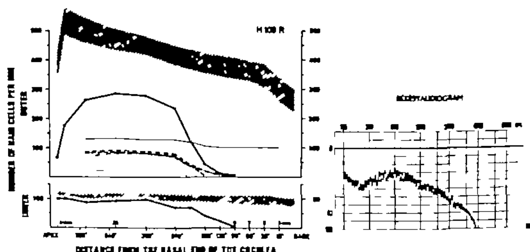


Fig. 53

Figs. 53 and 54. M aged 3 y s. N h st ry f noise xpos re ea disease A topsy diagnosi bronch l r neum heum tic endocarditis, d acute heart f il re

The cochl as ho l lmost omplet degenerat on of nerv f bers and of the rgan f Corti in the l w ba l l (right cochlea, Fig. 53). Inne nd outer hai cell pro nounced degenerat in N mb f ha ll right out 13 3; i ner 1900; left ea

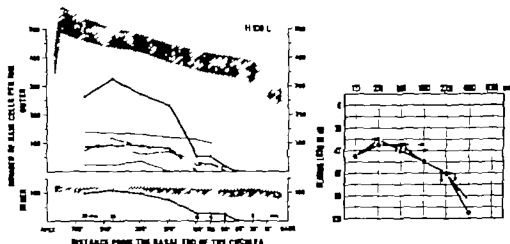


Fig 54

male 50 10; lane 1850 Length of the rgan of Corti right, 33.8 mm left, 31 4 mm. Pure tone audiogram f both ears is how l Fig. 54. Békésy audiogram of the left ear was similar to that of the right ea shown in Fig. 53. Speech discrimination score right, t left, 60 /o.

disappea In cochlea H 3 R (F'g 5) no piral bundles are e dent in th lower basal coil (0—180) and no fibers cross the re of compl te degenera tion f the radial fibers. In nother cochlea (Fig 57) sh wing a large area of t t l loss of both r d l fibers and organ of Corti, several bundles of spiral fibers are seen to run th ugh th correspond ng reg on of the osseous spiral lam n

4 *Relati n between lesions in the o gan of Cort and d gen rat on of n rve fibers i the osseous pi l lamin*

A complete loss of both inner and uter h ir ells in wid r narrow segment f the organ of Corti was n ariably associated with an bsence of radial nerve fibers in the corresponding sector f th osseous p ral lamina This was bserved t the basal end of every cochlea stud ed Othe examples were found n cochleas sh w ng areas f ircumscribed degen ration (Figs 3 –5 83)

A complet loss of, r reducti n in density of outer hair cells only was never ssoci ted with a not ce ble rarefact on loss of radial nerve fibers in th co respond ng sector of the osseous sp ral l m na This relation is well llust ted in cochl a N H 10" L (Fig. 58) showing large ea f exten- lo f oute hai cells, whilst the inner ha cells are nearl intact. The nn rv t n d n t n the correspond ng sector of th osseous pi l lamina does n t l ff n t ce bly from that f neighbouring regio s which show con d bl l gher den ity of outer hair cells

A complet loss of, o a reduct n in the number of n h cells in a

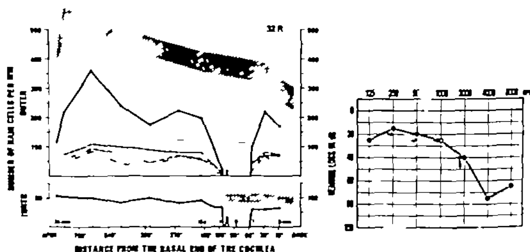


Fig. 53. Cochlea from ... aged 3 ... No known history of noise exposure ... disease ... diagnosis ... carcinoma ... larynx. Irradiated and treated with cytotoxic drugs. The cochlea ... areas of total degeneration both in the nerves and ... the organ of Corti. ... between these areas ... bundle of nerve fibers run out to the organ of Corti which ... inner hair cells and ... very few outer hair cells in the corresponding area. ... areas of total loss of sensory cells the outer hair cell population ... an overall ... which is accentuated ... the region between 360 ... and 510 ... Number of hair ... 000 ... inner ... Length of the organ of Corti, 33.0 mm.

Acta oto-laryng. ...

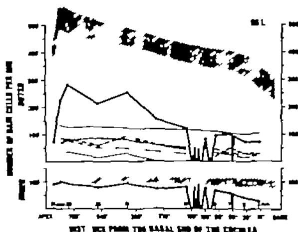


Fig. 56. Cochlea and graph of sensory cell population from woman aged 86 years. Autopsy diagnosis: cirrhosis of the liver.

The cochlea shows overall degeneration of nerve fibers most marked at the base. Outer hair cells show degeneration. Moreover there is patchy degeneration of nerves and the corresponding areas of the organ of Corti (arrows). Number of hair cells: outer 4350, inner 2000. Length of the organ of Corti, 32.0 mm.

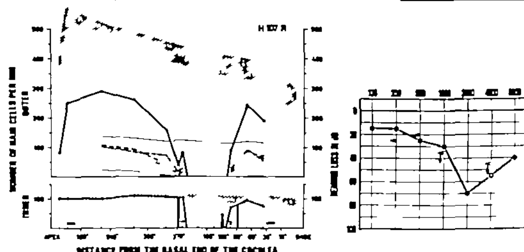
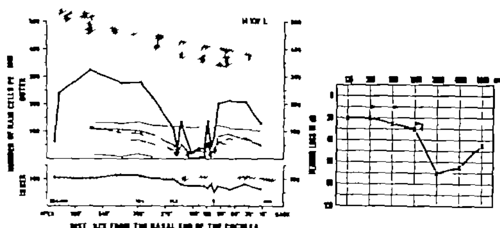


Fig. 57. Right cochlea from man aged 1 years. Exposed during lif t high I tensity noise in ea mill f many years. Aut psy diagnosis carcinoma of the stomach. Treated with t tal dose of 6 g. Streptomycin.

The cochlea show lmost complet degeneration f radial nerves i the osseous spiral lam as w ll corresponding degeneration of the rgan f Corti the region betwe 135 and 170 (10.5 mm—11 mm) Only few thi radi l nerv bundles re found in this re f complet degeneration (black rrow) I rel ted segment f the rgan of Cort few uner h i cells re present wherea ll out h i cell ha degenerated. Note that some sp rall running nerv bundles pass through thi re of the osseous spiral lamina I adid i on t th broad rea of gross degeneration there re three rrow rea showi g nerv loss (white rrow) I the correspond ng regions of the rga of Cort the i ne ha cell re m ss g There l an ov rall ppe rance f sl ght trophy in the radi l nerv fibers throughout N mber f ha cell out 11 0 inne 150 Le gth of the rga of C rt 31 mm.

The pure ne and ogram compares w ll w th the ha ges in the cochlea.



F g 58. The lef cochlea from the same subject as in Fig. 57 The udiogram sho be rm loss simil t th in the right ea Note the striking difference between the tw in be ppearanc of the nerv bundles in the osseous spiral lamina, ith no circumscribed degenera ion in th ea I the population of oute hai cells, however this ear hows pronounced degeneration in the region correspondi g to that of t tal degeneration be right ea Note that the inne hai cell how lmost no degeneration in this rea of be rgan of C rt Number of hai cells outer 5510 inner 2190 *Length of the orga of* ort 31 8 mm

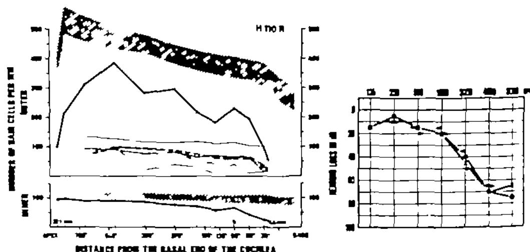


Fig. 59

Figs. 59 and 60. Right and left cochlea from man aged 81 years. No history of noise exposure or ear disease. Autopsy diagnosis: cancer of the oesophagus. Treated with a total of 1... g. Streptomycin.

Both cochleae show *pronounced patchy degeneration of nerve fibers throughout all coil.* The sensory cell populations are well preserved for the ... and do not show ... patchy degeneration. Number of hair cells: right ear, outer 283, inner 2205; left ear, outer 698, inner ...60. Length of the organ of Corti: right, 33. mm; left, 33.8 mm.

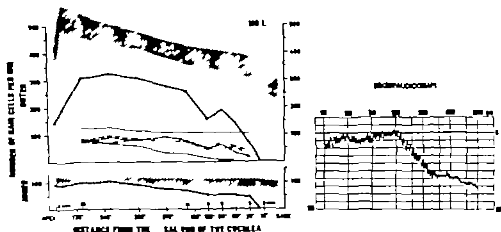


Fig 60

The pure one udiogram for both ears is show in Fig. 59 The Békésy audiogram f the right as simila t that for the left shown i Fig. 60 There i diminished differ-enc limen f he frequencies of 2000 cps nd abov The speech discriminat on score was f he right ea and 81 / f the left. Speech threshold were 3 and 25 dB res-pect l

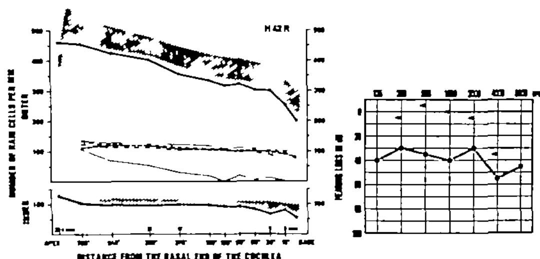
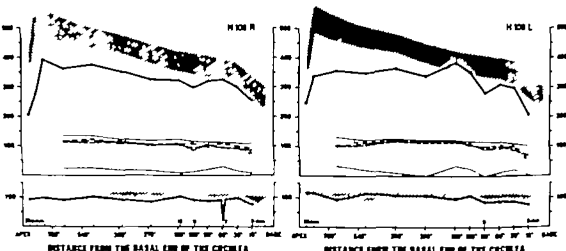
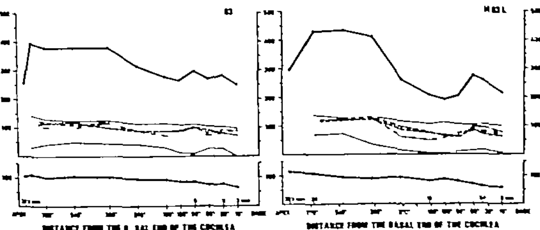


Fig. 61 M ged 18 years. N hist ry of noise posure ea disease Autopsy di g-nosed anaplastic carcinoma f the nose Treated with irradiation and cyt t ic drugs.
Outer hai cell light, diffusely-spread d generation th roughout all coils. Several early st ges of rep ir (collapse f gures) were found (see Fig. 9). In some places, groups of sensory nd supporting cells were degenerated (see Fig. 41) A few inne hai cell were lost. Number of hai cell te 11935; inner 3080. Length f th rga of Corti 33.8 mm. N rv supply i the osseous spiral l mina norm l
The pure tone audiogram hows conducti hearing loss which w simil for both ears. Bone conduction lmost norm l Th middle ears were filled with transud te A udiogram btained si months earlier howed rmal i conduction. Speech discrimination score 9 s.

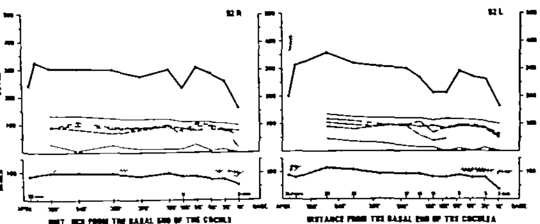


F gs. 62 and 63 M ged 35. N hi t ry f noise posure ea dise se Autopsy diagnosis diabetes mellitus with neuropathy retinopathy nd ren l f il re Outer ha cell l ght overall degeneration whi h is more accentuated towards the pex. I the right (Fig. 62) the inne ha cell re lmost complet ly degenerated i narrow region (1 1 mm w de) t 60 (mm) from the bas l end. N rv fibers the osseous spiral lamina normal throughout both cochleas. Number f ha cell right ea outer 10 40 inner 3070 left ea out 10100 nner 31 5. Length of the rgan of Corti right, 31.8 mm left, 31 0 mm Pure tone audiogram is shown. Speech discrimination scores were normal i both ears.



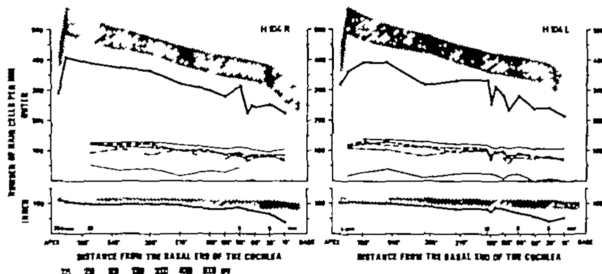
Figs. 64 and 65. Man aged 29 years. During lif exposed to high intensity noise in ship-y rd for sev ral years. Autopsy diagnosis bronchial carcinoma. Treated with ...l Send xan.

Right ea (Fig. 64) overall reduction in density of outer hai cells. I the region between 135 and 70 (11—1 mm), the degeneration is slightly more pronounced Left (Fig. 65) pronounced circumscribed degeneration of out hai cells i the region bet een 90 and 70 (9—1 mm). I thi rea the third row outer hai cell is more damaged than the other rows. N mber of hai cells right ea oute 10310; inner 3020; left ea oute 9790 inner 2815. Length f the rgan of Corti right, 31.. mm; left, 33.3 mm



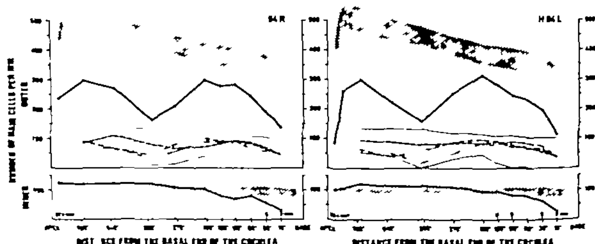
Figs. 66 and 6 A 53 yea old mechanic who f several years was exposed t high intensity noise in workshop. Autopsy diagnosis bronchial carcinoma. Tre ted with total dose of 4.6 g. Sendoxan.

Right ea (Fig. 66) ov rall degeneration of oute hai cells hich is slightly more pronounced towards the apex. Also in circumscribed regio t 135 (11 mm) the degenera ion wa more severe Left ea (Fig. 6) simila findings but the circumscribed degeneration of oute hai cell was slightl more pronounced t 135 (11—13 mm). The third row was more dam ged tha the other rows. There were no majo changes in the nerv supply in the osseous spiral lamina. Numbe f hair cell right ea outer 8850 inne 2850; left ear outer 9320 inner 2970 Length of the organ of Corti right, not possible t measure; l ft, 35. mm



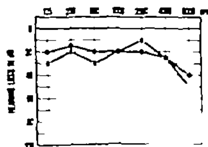
F gs. 68 and 69 M ged 55 y rs. During lif exposed for ma y y rs t industrial noise Autopsy diagnosis pulmonary carcinoma. Tre ted with cytotoxic drugs.

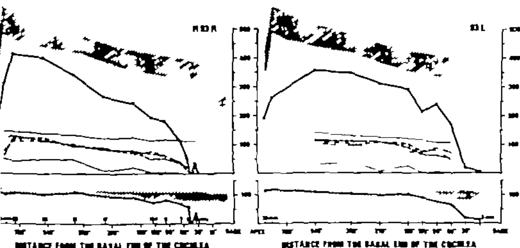
Right (Fig. 68) nd left cochlea (Fig. 69) overall loss of outer hai cell I the region between 60 nd 180 there occu local ariations i density f cells I ne hai cells moderat basal loss. Nerv fibers in the osseous spiral lamina no pparent cha ges. Number of hai cell right ea outer 1011 ; inne 28.0 left ea outer 9380; inner 2560 Length f the rga of Corti right, 31.3 mm; left, 3..9 mm.



F gs. 0 and 1 M aged 62 y rs. Exposed f ma y years d ri g l f t noise i the engine oom f diesel motor hip. Autopsy diagnosis pulmon ry carcinoma. Treated with dose of 1.6 g. Send xan.

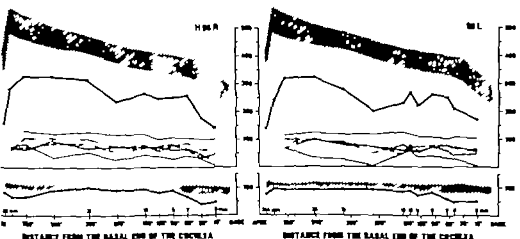
R ght (F g. 0) nd left cochlea (Fig 1) overall reduction f the density f out hai cells nd i dd'tion wide circumscribed rea. of more pronounced loss between "0 and 10 (1 – .3 mm). Inner h i cell moderat basal loss. Not that lthough the majo loss f out hai cell was found the middle and apical coils, the hearing loss for the h gh frequencies l sl ghtly m re pronounced tha f the lo er frequencies. Number of ha cell right ea outer 120 nne 30"5 left ea outer 0.. inne 3023. Length f he rga of Corti right 33.3 mm left, 33 (mm





Figs. 2 and 3. Man aged 63 years. Exposed during life to high intensity noise in shipyard for many years. Autopsy diagnosis bronchial carcinoma. Treated with dose of 7.4 g. Sendoxan.

Right cochlea (Fig.) mainly basal degeneration of both inner and outer hair cells. Left cochlea (Fig. 3) similar findings but slightly more pronounced overall degeneration of the outer hair cells. Number of hair cells right ear outer 310; inner 2510; left ear outer 8000 inner 2‾‾5. Length of the organ of Corti right, 32.2 mm; left not measured.



Figs. 4 and 5. Man aged 61. No history of noise exposure. Diagnosis bronchial carcinoma. Treated with 6 g. Sendoxan. Nerve fibers in the osseous spiral lamina complete loss in the basal 2 mm density gradually increasing up to 180 (13 mm). Several minor areas of nerve loss occur together with loss of a few inner hair cells in corresponding segments. In the apical coil an area of accentuated nerve loss. Sensory cells Outer: overall loss, more pronounced at 2‾0 (1 mm). Inner: basal and apical loss. Number of cells right ear outer 83‾5; inner 2625; left ear outer 180; inner 2110. Length of the organ of Corti right, not measured; left, 32.5 mm. Audiometry conductive hearing loss in the right ear (large dry perforation of the tympanic membrane). Speech discrimination score 6 / 5 both ears.

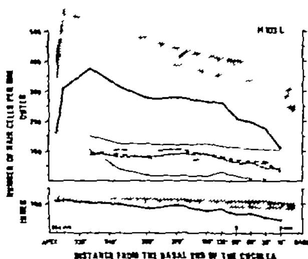
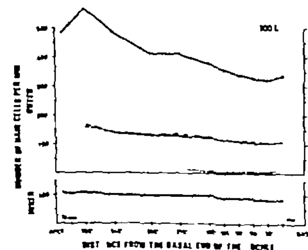


Fig 6

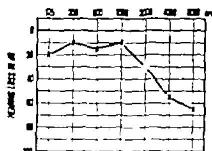
Fig 6 M ged 0 y s. N hist ry of noise exposure r ea disease Autopsy diagnosis bronchial carcinoma. The cochlea how mod rat basal degeneratio of nerv fibers i the osseous spiral lam a. Outer hai cell : overall degeneration, slightly more pronounced toward the basal end. Inner h i cells mod rate basal degeneration. Number of hai cell outer 8370; i ne 26 0 Length f th rga f Corti, 31.6 mm.

F g — Popul tion of sensory ll f the rg of Cort from newborn, f llterm hild who died of acut birth tra ma. There i ign of degeneration f sensory cell i thi cochlea. N t that the density of cells incides w ll with that f the f t l material (the striped reas) Number of hai cell oute 13850 inner 33_5 *The length of the rg of Corti wa not* meas red



F g —

Fig *8 and 9* Boy aged 5 years. Autopsy di gnosi leucemia. Treated f tw years with corticost reoid d the cytot xic drug p rinethol The sensory cell popul tions show in both ears (right, Fig. 8; left, Fig. 79) slight degeneration of out hai cell i the middl coil. I the right the outer h i cell were complet ly degenerated in the region between 5 nd mm f om the bas l end. This degeneratio showed the early stages of repai (cell pse figures) indicati g that the lesion occurred not too long bef re death. Numbe of hai cell right ea outer 12"30; inne 31 0; left ea outer 13120; i ne 3140 Length of the rga of Corti right, 36.0 mm; left 36.6 mm.



Figs 80 and 81 Woman ged _3 y rs N hist ry of noise exposure ear disse A t psy diagnosi carcinoma of the ll with metastases. Tre ted with cytot ic drugs. The sensory cell populations sh w i both rs (right, Fig. 80; left, Fig. 81) slight ov rall degeneration of oute hai cells. M y earl signs of repai (cell pse figures) N mbe f hai cells right e outer 118"5; i ne 3000; left out 10620; inner 2960 Length f the ga f Corti; right, 33 1 mm; left, 33.5 mm.

F g 82 M aged 31 years. A topsy d agnosis intestinal bstruction (strangulation of the mesent rial root). The oute hai cell how mod rat basal nd pical degeneration only Number f h ir cell out 11880 i ne 32"0 Length of the org of Corti, 33.6 mm.

Fig 83 M ed 31 y rs. N history of noise posure ea disease Aut psy diagnosi m l gnant tum of the testi with metastases. Tre ted with cytoto ic drugs. The cochlea he a circumscribed region of complete degeneration of the ga of Corti nd the nerves i the corresp ndi segment of the osseous spiral lami a. The outer hai cell ov rall degeneration, m re accentuated t w rd the base Number of h i cell outer 9330 inner 2"00 The length of the orga of Corti w not measured.

(see also *p* *36*

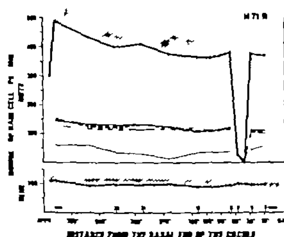


Fig. 78.

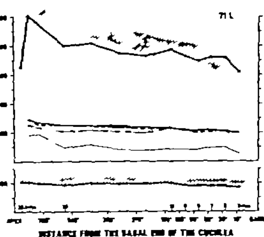


Fig. 79

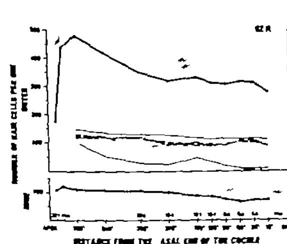


Fig. 80



Fig. 81

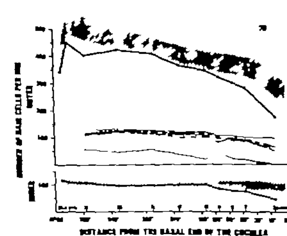


Fig. 82.

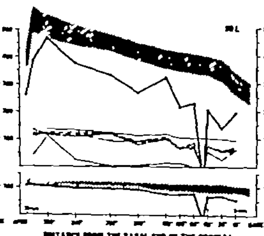


Fig. 83

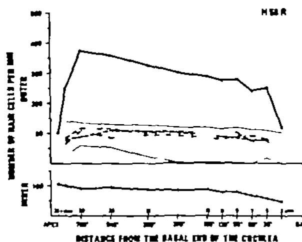


Fig. 84

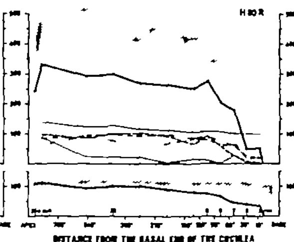


Fig. 85

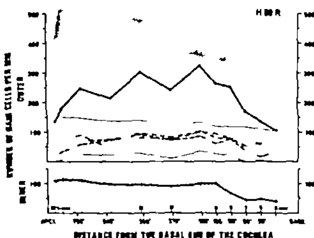


Fig. 86.

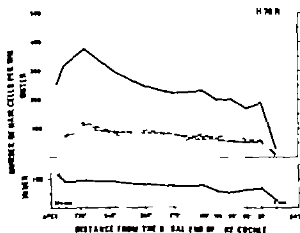


Fig. 87

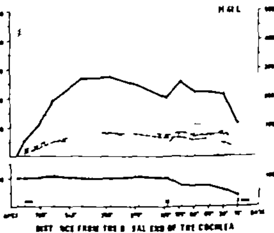


Fig. 88

regi n of the organ of Corti with intact outer h ir cell wa with one excepti n, correlated with a loss or rarefacti n of the nerve fibers in the corresponding sector of the osseous sp ral lamina Thi pattern of degeneration was observed in bout 0 s tuat ons, in 10 cochlea The lesion were less than 1 mm in length usually 0 1—0 4 mm Often a rarefacti n of nerve fibers wa noticeable in zones corresponding to a loss of onl 3—4 neighbouring inner hair cells The supporting cell were generall normal The ingle exception to this pattern was observed in cochlea No H 108 R (Fig 6*) how ng a circumscribed zone of degeneration (about 0 4 mm in length) of the inner hair cells, in which few scattered cells rema ned intact The nerve fibers showed normal density

Another aspect of the relation between nerve fibers and hair cell i illustrated n cochleas show ng a patchy degenerati n of nerves and organ of C rti (e g Figs 55—5" 89) The small intact area of the organ of Corti sur rounded by completely degenerated areas, are supplied each w th it own bundle of rad al nerves The outer l air cells are almost completely legenerated whereas the inner ha r cells ha e su t ined relatively les pronounced damage In cochlea H 10 R (Fig ") there a large d generated area w th n wh ch were two sm ll island w th respectivel two and tl ree inner h ir cells Even these l ad small bundles of 15 and 20 nerve fibers respectively

Fig 84 Woman aged 68 years. N hist ry f noise exposure ea disease Autopsy diagnosis carcinoma f the l rge intestine The nerv supply in the osseous spiral lamina sho moderate basal degeneration and more pronounced degeneration in the pical coil. Outer hai cells overall degeneration. Inne hai cells basal degeneration. Number f hai cell outer 9075 inner 2700. Length of the rgan of Corti, 31 1 mm.

Fig. 85 Man ged 68 years. N hist ry f noise exposure ea d sease A topsy diagnosis cardioatherosclerosis, acut he rt fail re nd di betes mellitus. The nerv supply i the osseous spiral lamina how an ov rall rarefaction, more accentuated tow rds the basal end. Oute hai cells overall degeneration which i more pronounced tow rd the basal end. Inner hai cells basal degenerati n. Numbe of hai cells outer 8515; inne 2960 Length of the organ of Corti, 37.6 mm.

Fig 86 Woman ged 69 years. N hist ry of noise exposure or ea disease A topsy diagnosis bronchopneumonia and therosclerosis. The nerv supply in the osseous spiral lamina shows an overall rarefaction which is more pronounced t rd the base Outer hair cells degeneration which increases from the poi 180° tow rd both the basal and the apical ends. Inne hair cells basal degenera ion. Numbe of hai cells outer 315; inne 2735 Length of the rgan of Cort 33 5 mm

Fig. 87 Woma aged 3 years. N h st ry f no se exposure ea disease A topsy diagnosis gangrene f the small intest ne and cardioatherosclerosis. The nerve fibers i the osseous spiral lam sho light ov rall rarefaction, m re pronounced tow rds the base Oute hai ll ov rall degenera on. Inner hai cell moderate degeneration, more accentuated tow rds the base N mbe of ha cells oute 130 inner 238. Length of the rga of Corti, 33 8 mm

Fig 88. M aged 8 N h st ry of ise exposure or ea disease Autopsy diagnosis carcinoma f he oesop treated ith cyt t ic drugs. The nerv fibers in the osseous spiral lam na show mod er ll degeneration, accentuated tow rd the base Oute hai cell overall deg ne wi h increases tow rds the apex. Inne ha cell moderate basal degeneration Numl of h cell outer 6660; inner 2890 Length of the rgan of Corti, 33 1 mm



Fig. 89 Osseous spiral lamina and associated basilar membrane and organ of Corti of an 86 year old woman (cochlea II 55 I Fig. 56) surface preparation half coil (11 mm) from the basal end. In the region of the specimen the radial nerve bundles running in the osseous spiral lamina are rarefied and the organ of Corti in the corresponding regions is completely degenerated leaving a bare basilar membrane (Magnification 80)



Fig. 90 Osseous spiral lamina and associated organ of Corti of an 80 year old man (cochlea II 110 R Fig. 39) surface preparation from the upper basal coil (1 mm). The radial nerve bundles in the lamina show patchy degeneration. In the area to the left the bundles are completely missing. The appearance of narrowing in the corresponding part of the organ of Corti (arrow) indicates atrophy of the supporting cells between the outer hair cells and the sulcus term (Magnification 80)

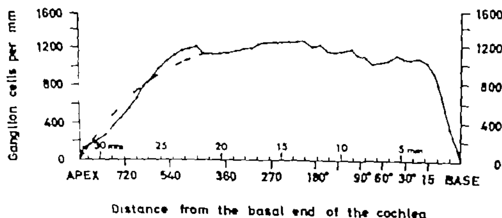
running out to them In thi instance the outer hair cell were completel degenerated and the supporting cell exten ively so

In one pair of cochlea (F g 59 60 90) loss of nerve fibers wa not associated with a corresponding loss of sensory cells. These cochle s showed extens ve patchy degeneration of the nerves throughout whereas the sensory cell populations fa led to show corresponding gaps

D scussi n

The fetal cochlea was used in th s tudy as ref rence base for comparison and estimation of the cell degeneration in the postnatal cochle This may ppea questionable t some but there is certain justif cation and certain reasons for relying on the fetal cochlea as a control The justificat on rests primarily on the l ck of ny other cl oice The fetal and newborn cochle s were the only ones seen wh ch were normal in the sense f not sh wing degenerated h i cells. As discussed in det l in part A of this hapter loss of sensory cells apparently does n t occur normally in the fetal cochl a The full complement f sensory cells seems to be attained late in th fourth fetal month th only h nges wl ich take place the e fte be ng n the nature of w dening of the organ of Corti concom tantly with the development of fluid spaces Th sensory cell populat n of the newborn h ld (F g) grees w th that of the lat r f t l cochle

It must be emph sized that the reference le el n the graphs (the hatched areas) how the range of ariat on in the numbe of cells pe millimeter length f th organ f Corti as estim ted in sev n fetuses A this variat on is fairly large, nd th number of cochleas small, statistical analysis would not nhance the ignif cante of tl e d ta Accordingly cochlea m y show a



F g. 91 Dens of ganglion cells pe mm length of the cochlea (*Redrawn fter We* 1949 Theory f Hearing, F g 82)

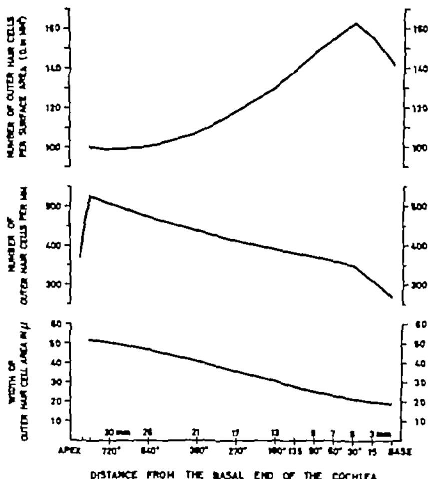


Fig. 92. Graph representing (from above) density of outer hair cells per surface area, number of cells per mm length of the organ of Corti, and the width of the outer hair cell area. Note that the density of cells per surface area has its maximum at the base whereas the number of cells per mm has its maximum at the apex. The graph of cells/mm represents an average of fetal cochleas. The width of the outer hair cell area average of three cochleas from young adults.

full complement of cells and still the curve may lie outside the hatched area. However in the first row of outer hair cells the full number of cells can be estimated (see page 1) and accordingly one can evaluate whether or not a loss of cells in these three rows occurs.

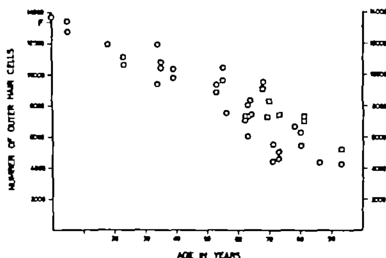
The innervation density as measured by ganglion cell count has its maximum in the upper basal coil and decreases toward the apex (see Fig. 91) (Guild 193 , Wever 1949, Schuknecht 1960). It has been suggested that this density distribution of innervation is related to threshold sensitivity for pure tones. Threshold sensitivity is maximal at about 2000 cps and diminishes for lower and higher frequencies. This does not seem to be in accord with the observation that the hair cell density as measured by cells per millimeter length of the organ of Corti increases toward the apex (Fig. 9). The number of afferent n l g a l outer hair cells is considered to be uniform

or even to increase toward the apex (Smith and Sjostrand 1961) thi w uld imply either that the afferent nerve fibers in the middl and pical coils make contact with a considerably larger number of outer hair cell than they do in the basal coil o that they supply each cell with sev ral nerve endings

Another way of measuring sensory cell den ity is in terms of the number of cells per surface area Thus the density of the outer ha r cells in the area of contact between the hairs and the tect rial membr ne has its maximum at 30° (5 mm from basal end) and decreases towards the apex (Fig 9?) This density parameter may be of import nce n the mechanical stimulation of the ha r cells. The spreading of the hair cells over larger area (the d s-tance between the inner hair cell row and the f rst outer row also increases tow rd the apex) is paralleled by an increase in width of the bas lar m m brane and s ze of the organ of Cort includ ng an increase n he ght of the sensory cells and th ir hairs

The total number of cells in each cochlea plotted against th age. (Figs 93 and 94) gives no detailed information as to regi nal diff rences of distri bution of ha cell loss through ut the cochlea neverth less. this graph is a good starting point for discussion of cochlear damage a related to age ng The cell counts, in general seem to show a tenden y to linear reduct on w th increasing age lth ugh the range of variat on is large especially o er the age of 50 The more extens ve loss of ut r hair cells tl an of nner agrees well with the general op ni n that out r h ir cells are more ulnerabl than the inne F en in the first and second decades of life loss of outer sensory cells becomes n ticeable This is rem rkable when one considers the fa t that maximum sens tivity to thresh ld stimul by pure t nes. and th maximum audible frequ ncy range are att ined during the second decade of l fe Like-wise the ea ly loss of cells in the cochle is n tabl in comparison w th th later ppearance f cell degen ration in the bra n where norm l degenera-tion begins after the age of 20 (Brod 1955) All of the subjects n th present material between the ages 5 and 23 years suffered from malignant tumors and were tre ted for 6—29 m nths with cytotoxic an lges nd other drugs A cert in amount of recent degen rati n of the sensory cells (collapse-figures) was observed in these cochl as so that it may be possible that th cell loss found can be the result of a tox c effect Accordingly it is possible th t no majo damage has been demonstrated o is n rm lly to be expected at those ages

In contrast to the igns of recent dam ge in the y ung subjects, no such signs w re bserved in subjects old than 30 ye rs. alth ugh most f th m lso suffered from m l gnant tumors and w re treated with the same kind of drugs. Thus, it seems th t th young cochlea may be more vulnerable or the toxic influence m re severe in the young subjets Whatever the reason for this difference in ochl ar d m ge t seems th t the cochl s in the over-30 ge groups w les nfluenced by the t rm n l disease nd re thus m re representative of the norm l tructure of ach cochlea than re those of the younge grou



F g 93 R latlonsh p between t t l numbe of out h i cells nd ge F indicates the f ll complement f cell as obt ined from fet l m t ri l The squares represent cochleas f om norm l he ri g sul ject

Pure t ne au liogram f r ears included in thi study were compared w th normal hearing for the age a alculated l y Spoor (1967) in his study of 8 audiometric investigati n of presl ycusi in order to determine to what extent the cochlea may be con dered representati e for their age It is evident that the majority of au liogram in the present investigation how threshold f r pure tones which are higl er than those of the c lculated presl ycu i curves Several of the audiogram also have a different shape even th ugh they may how normal or better hearing f r some frequencies Nevertheless, four of the cochlea did produce audi gram typical for their age Several other am ng the audiogram prol al ly ll within the normal range of aria t on but were exclu led becau e all showe l threshold lightly higher tl n tl e calculated averag (Spoor 1967) The f ur norm l for age cochl a (Figs 1 9 60 6) all ha l sensory cell population whi h fit into th upper part of th l tribution curve for outer hair cell count Thi regi n of the curve also ncompa ses the cell count for three cochl a from femal subject who h l no hi tory of h gl inten ity n ise exposure o ear lisease (Fig 84 86 8) Tl us, there i an apparent tendency for the cochlea which are nor mal for the ag to form the upper porti n of the age/cell popul t on curve Tl i group of coel l a i furtl er characteri ed by tl f t tl t th ir inn r l a r cell cou nt are not m ural ly liff rent fr m those of cochle with m re pron nced l aring impairm nt ind cating tl at tl number of inn r h r cell n thes ases loes ot correlat witl thre l l l sen iti ity f r pure tones

It must be r m mbere l tl at th tl resh l l f r pure t nes i only on of the par m t rs n the ompl l nical picture of presb cu i a con d ration whi l m l t t f m tl alidit of the comp r son m le in the f re

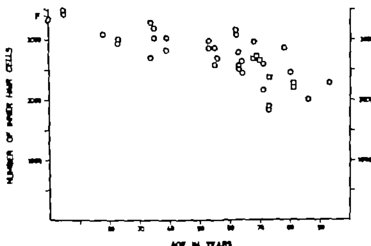


Fig 91 Rel tionship between t t l umbe f inne hai cells nd ge F indicates the full complement of cell as btained from fet l m t ri l The squares represent cochlea from normal-hearing subjects.

going p ragraph It is also import nt t be r in n in l that the term presbycu i is used here in the context of th presbycu i urve for modern c vil zation and that the cochleas of other populati ns, such as the Mabaans (R sen *et l* 1962, 1964) might h w a complet ly different picture of sensory cell popul tions It is apparent th t the only w y t resol some of th pen questio is by th study of m ny more cochleas from sul ject of all ages in wh m the hearing has been tested th rougl ly and all causes f he ring loss tl er than presbycusis hav been e luded

The l terature contains few repo t which con incingly ttribut mor phol gical changes in the organ of Corti to ageing Tl e present nvestigati n indicates th t the compressi n f tl e organ f Co ti in tl e angiosclerotl d generati n of v Fie ndt nd S xén l an rt fa t (see page 47) Several studies have described a m re or less pronounced atrophy f th organ f Corti in the low b sal coil (F l ny) 1931 Crow Guild nd I olv gt 1934 and others) but the rel tion to ageing has n t been clear Fl isch r (1956) n fa t pressed d ul t th t su h rrel ti n could be ma l Schu knecht (1955 1964) reported n atrophy f the rgan of Corti an l th nerve in tl e most basal f w m ll m t rs tl e tructural correl t f n type f presbycusis A corresp nd ng k n l f h nge i f un l in alm st all f tl cochl as in tl present mat ri l

H ret f re reduction of sensory cell pop lati n witl ut atrophy f the organ of Corti has n t bee described a relat l t ag ing In fact many report t t th t n se ral hlea from very old peo le the sensory cell popul t ns w re int t Schuknecht (1967) t teds Clini al xperience r v l mmon type f se sorineural l aring loss wl ich i lowly progre ive

during the latter decades of life and i characterized by a descending audiometric curve Thu far all attempts by light microscopy to find a structural correlate have failed In the present tudy however there is found a decrease in the number of sensory cell with increa ing age Thi reduction in the hair cell population seem to be a con tant finding and has a clear relation to age (Fig 38, 93 91) Thi loes not exclude that other factors may al be of importance in thi relation

It i worth noting that the basal reduction of both inner and outer hair cell occur while the general shape of the organ of Corti i still normal It i only when the cell los i almost complete that general collapse occurs The apical l ss of outer hair cell wa never associated with a general atrophy of the organ of Corti Although degeneration of nerves and ganglion cells in the apical coil have been described earlier (Guild 1935 Covell and Rogers 1957 Lindsay and v Schulte 1958 Bern tein and Schuknecht 196) no mention ha been made of a specific leg neration of sensory cells in thi region

The mo t nearly con tant hi topathological finding in the cochlea in ol l age i an atrophy of the spiral ganglion and the nerves in the osseous spiral lamina (Brühl 190 Mana se 1906 Fabinyi 1931 v Fieandt and Saxén 193 Fleischer 1956 Schuknecht 19 5 1964 Jorgensen 1961; Hansen and Reske-Nielsen 1965 Makishima 196) These descriptions correlate well with the los of nerves in the o seou spiral lamina seen in the present material Fleischer (1956) analysed 100 temporal bones from subject 0—90 years ol l and correlated a reduction in den ity of ganglion cell corresponding to the lower basal coil with increa ing age In the present study both the nerves and the sensory cell in the lower ba al coil seem to undergo about the same degree of reduction with ageing a that illu trated by Fleischer's ganglion cell count

From the discu ion above it i evident that a discrepan y exi t between previously described morphological changes in the organ of Corti and those of the present investigation Thi i prol al ly a result f difference in techniques used, whi h will be d scusse l in a lat r hapt r

Schuknecht (1953 l 1964) con i lered d generation of ganglion cell an l n rves in tl e osseou piral lamin to be more crucially affected by the extent of injury uff red l y th supporting cell of tl organ of Corti p rti ul rly th pillar cell th n b l mage to the sensory cell He uggested that it wa a tuall possil le for all the sensory cell in a particul r region of the cochlea to disappear whil th corresponding piral ganglion cell remained normal (Schuknecht 19 3 l) It must be empha zed that those ol servati n were l sed on experim nt lly produced lamage in anim l In th present study it wa foun l that comp l te l ss of sensory cell in a giv n segment wa invariably accompanied l complete los of n rves in correspond ng segment of th osseou piral l min Furth rmore sev ral area we ol rved to have ustained a severe l ss of p ll r cell with ut a correspon l ng lo f n rve uppl On tl oth r han l wit ngl exception tl n rves w re lost or co n

nderably rarefied in areas corresponding to a loss of inne hai cells In two small islands of the organ of Corti surrounded b large areas of complete degeneration, two and three inner hair cell respecti ely were intact, whereas all outer h ir cells were lost About 15 and 20 radial nerve fibers, respect vely supplied th se areas As there is no evidence that th afferent nerves in the osseous spiral lamina can survive if the organ of Corti is completely lost, it seems probable that those fibers made connection with the inner hair cells This would mean that each of these inner hair cells was innervated by at least radial fibers Thus the present evidence bespe ks a close correlation between inner hair cell loss and nerve loss. As for the outer ha cells, a severe to total loss of these cells in one region was ne er ssociated w th any noticeable rarefaction of nerve fibers in the correspond ng sector of the osseous spiral lamina, provided that the inner hair cell remained This is illustrated n cochlea No H 10 L (Fig 58)

One possibl e planation of this pattern of degeneration would be th t the inner hair cells are much more richly inn rv ted than the out r nes Accord ngly a loss of outer hair cells, supplied w th relati ely sm ll contingents of nerve fibres would cause a degenerati n of nerves which by the present method f study might not be not ceable whereas a loss of inne hair cells would cause consp cuous change in the nnervat on d nsity in the seous sp ral lamina Such an hypothesis is contradictory to most prev ous studies f the innervati n of the cochle (Retzius, 1884; Caj l 1909—1911; Lorente de N 193 Fernandez, 19 1) h weve most previous descripti ns nd cate that nne hair cells are supplied by radial fibers which run directly to the cells, whereas the outer hair cells are largely nnervated by sp roneurones wh ch run p rally for unknown distances, a h fibe innervating many oute hair cells

Accordingly any nerv degen rat on resulti g from outer hair cell damage should be diffused o er a widespread zon of the osseous spiral lam n and hence would not be conspicu us Furth rmore since the sp roneurones are known to have endings on many oute hair cells, distributed o er con siderable linear distances the statistical ha ce that all f such a group would be ffected by ny one lesi n s sm ll This rgument is based on the assumption that the relat ns between hair cells and nerve endings are l k synapt junctions lsewhere th t is, that in cases where multicell lar connect ons are the rul many of those may be destroyed, but so lo g as cert n numbe are intact, the nerve m y be ust ined

In summ ry the p ttern of degen ration described in the present study does n t necessarily suggest th t the uter h i cells re provided sp rsely with ff nt f bers. On th l e h nd, the findings do ind cat close inter depend nce between inner h i cells nd radial n rve fibers This could be in greement with the p ni n of Spoendl n (1966) He described tud es on th cat in which the efferent n erv tion w s sect ned, and found that the gre test number of fferent nerves end n th nne h i cells only bout n tenth extending to th ut r ha r cells Th present study is less than

crucial in thi sense a it wa not pos ible to differentiate between efferent and afferent innervation; hence conclusion mu t be correspondingly cautiou

One important con ideration in thi discu ion i that degeneration of sen sory cells can occur within a very short time after damage (probably less than one day) wherea it i generally accepted that complete disappearance of nerve fiber takes a longer time Lurie Davis and Hawkins (1944) found nerve fiber degeneration 9 day after noise expo ure and loss of ganglion cell after 3—4 week In the present investigation no recent sign of sensory cell degeneration were observed in the cases immediately under discu ion After noise exposure of experimental animals, the formation of completely healed lesion in the organ of Corti takes at lea t a couple of weeks Accordingly thi factor i apparently not relevant in explaining the pattern of degeneration

It i generally accepted that a lesion to the organ of Corti which is so severe a to cause complete degeneration i a sociated with a corresponding complete los of nerves however the congenitally deaf Dalmatian dog i described a howing a different pattern of degeneration This animal ha an intact population of sensory cells during fetal life (Fig 95) but at some time during late fetal life or soon after birth the sen ory organ begin to degenerate Thi proces is reported to end with a complete compression and degeneration of the organ of Corti and its sensory cell wherea the nerves in the o seou spiral lamina and spiral ganglion remain relatively intact (Alt mann 19 0 Hudson Durham and Ruben 196 Anderson *et al.*, 1968) The author ha tudied three such completely deaf dog of 8—11 week of age u ing the surface pecimen technique Although the organ of Corti wa completely collapsed and the outer hair cells completely degenerated the inner hair cell remained largely present (Fig 9) It might be pos ible that in previous studies made on section the inner hair cell were not discernible in the compressed organ of Corti wherea they were di tinctly seen in surface preparation Accordingly it might be pos ible that the presence of inner hair cell *account f r the good supply of nerve in the osse u spiral lamina* Thi evid nce i less than conclu iv however a it i possibl that the Dalmatian dog studied by the author were too young to have gone through the com plete degenerative process Another con ideration i th at the mechani m under lying thi d generation i unknown and perhaps quite different from what causes the degeneration described in the human subject of thi tudy

One pair of cochlea in the present materi l showed a pron unced nerve d g n rati n which wa not a sociated with corresponding loss of hair cell (Fig 9 an l 60) It I been kn wn nce the beginning of thi century that after transecti n of the cochlear n rv the piral ganglion an l tl l n lrites n tl e osseou spir l lamina degenerate wh rea the org n of Cort rema n nt t Spoendlin and Gacek (1963) describe l electron microscopi tu l es i tl at which howed that even th aff rent n rv en l ngs remai l t rt aft r divi n of the cochle r nerve Tl se fin ling uggest that in tl hum cochle mention l also th n rve l gen r t n w the result

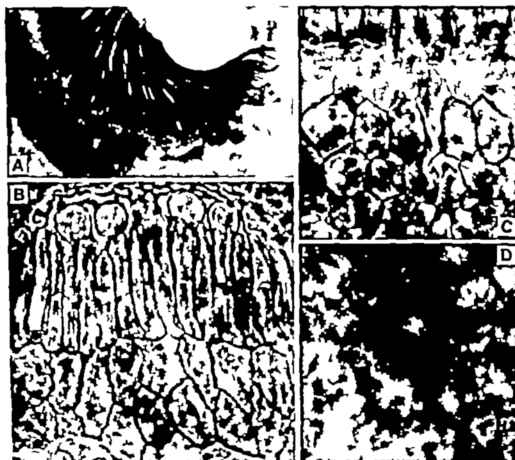


Fig 95 Cochlea of an 11 ek old congenitally deaf Dalmatian dog.

A Osseous spiral lamina from the pical coil showing dense nerv bundles, which were observed throughout the cochlea

B Organ of Corti, surface preparation from the lower middle coil, showing that the inner hai cells (IHC) re t large extent present, whereas no outer ha cells (rea between rows) re seen.

C Outer hai cell area (between rows) one coil from the basal end, showing absence of oute hai cells.

D The imprints f the ha bundles (H 1 H 2, H 3) as see in the tectorial membrane from the same region of the cochlea as in C, clearly indicate that the outer hai cells have once been present. (M gnification, A 1 B, C, and D 910)

of damage t th cochle neuron rathe than to the o gan of Corti In all other cochl as tudied, d mage to the organ of Cort cannot be cluded as th primary f t causing th nerve degen ration.

The distributi n f th spirally running fibers in the osseous spiral lamina described n th present study corresponds well with the distribution of efferent fib rs described by Gacek (1961) Ishii Murakami and Balogh (196) and N mura and Kirikae (196")

C. Comparison between cochlear morphology and hearing function

1 Threshold sensitivity for pure tones

Experimental evidence of several kinds over the la t three decades, ha led to wide acceptance of the idea that sound stimuli of diffrent frequencies have their maximum activating effects at different sites along the organ of Corti, each at its specific region It is generally agreed that high frequencies stimulate maximally the basal end, an l progres ively lower frequencies toward the apex There is said to be an almost linear correspondence between the logarithm of frenquency and distance from basal end to area of focal effect except for the highest and lowest frequencies (Bekesy 191_ Davis *et al.*, 1919 Schuknecht 1953 a) Thi would mean that each octave should be represented l y segments of the organ of Corti of about equal length Bekesy (1913) ha shown that although very l w frequencies are represented by specific regions of maximum effect these maxima are les sharply restricted in area than those of higher frequency stimuli An increase of the stimulus inten ity above threshold gives rise to a larger amplitude and also involves a larger area of the organ of Corti than a threshold stimulu This widening of the activated area i con idered to be more pronounced for low than for high frequencies.

In the present material it i evident that if we try t correlate the threshold for pure tones with the number of hair cells in a corresponding region of the organ of Corti great differences are found between region where l w frequencies and high frequencies are supposed to have their maximal represen tati n Thu a lo of a given number of outer hair cell in the lower ba al coil i related to a more pronounced pure tone hearing loss than is the loss of a similar number of cell in the upper middle and the apical coils. Moreover those cochlea with more exten ive apical than basal degeneration of outer hair cells still show more pron unced hearing loss for the high frequencies than for the low (Figs 51 5? 0 71) In order to analyze these relation more closely the h aring level in dB for the tested frequencies wa plotted again t th den ity of outer hair cell (cell/mm) in corresponding region (according to Bekesy 191?; Davis *et al.*, 1919 Schuknecht 1953 a) Two such diagram are shown in Fig 96 Mo t of the subject were however known to have had a hearing d fect during life For thi rea n the sound pres ure level require l to pr duce threshold stimulati n ha l to be greater than those nee led for n rmal hearing subject Such an increase must be atten led by ext n ion of th activated area for which reason it i not ompl t ly relevant t compare the hearing los with th len ity of cell in a narrow area of th organ of Corti Whether or not such an ext n ion may influence the relative threshol l sen iti ity i not known Theref re uch a rough comp rison a that mentioned abo e seem ju tified It i also neces ary to bear in mind that lesi n located centr l to the coel l a may also cause a thresh ll rise a indicated b animal periment l y Kr t r and Ades (1913)

A con ideral l l of outer ha r cell (up to 10 percent) may occur at 30 mm from l ase (- 0) wit out great l aring lo (1 dB or les)

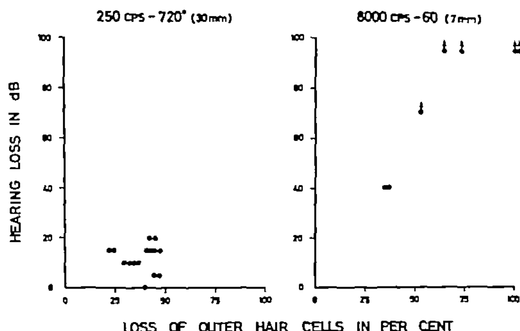


Fig. 96. Rel tionship between hearing loss and outer hai cell loss fo .50 cps— "0 (30 mm) and fo 8000 cps—60 (mm). The arrow indicate that the hearing loss fo these cases may be even more pronounced since the maximum stim lus intensity of the udiometers was not heard.

Several cochleas sh wed a loss of 50— 5 % of the oute h ir cells in the same regi n, the greatest hearing loss not exceeding 40 dB (Fig 96) On the other hand, a relatively much smaller loss of ute h ir cells at " mm from the basal end (8000 cps—60) was accompanied by pronounced hearing loss A 25 percent loss of out hair cells was rel ted to hearing impairment f .0—30 dB nd 50 percent cell loss to 50— 5 dB mpairment (Fig 96) Between these two extremes (.50 cps— 0 and 8000 cps—60) there was graded transiti n of the relati nship of cell loss t hearing loss. A deviati n from this grad ent was encountered at the 11 mm level (4000 cps—135) where a given loss of hair cells was associated with a lesser hearing loss than that produced by equal cell loss e the t 1" mm ("000 cps—2"0) or mm (8000 cps—60) This deviation may well be explained by the finding that in the present m terial th region at 135 (corresponding t 4000 cps) showed th highest incidence of c rcumscribed les ons in the organ of Corti Since these were mostly narrow n w dth, t is poss bl that neighbouring regions, sh wing less dam ge, were st mul ted rather th n the ctual regions normally reacting to a t ne f 4000 cps at thresh ld An illlustrative example f this is found in cochlea H 10 R (Fig.) which showed total degeneration of a 4 mm sect of th gan of Corti nd th n rves in the osseous sp ral lamina Th hearing losses f corresponding frequencies were n t more than 55— 0 dB

In cochleas showing circumscribed degeneration of the outer hair cell in the region of 2 0—360° no corresponding dip in the audiogram was observed (Fig 48 55 70 71) however hearing was tested only at octave interval and therefore one cannot exclude the pos il ility that some of these cochlea may have had a more pronounced depression of threshold sen itivity for a smaller frequency band in an interoctave interval

It was mentioned previou ly that the total number of inner hair cell in the cochleas with normal hearing for the age did not differ notably from that in cochleas showing more severe hearing impairment Hence in these cases the inner hair cells seem not to be related with threshold sen itivity The two ears of one subject (cochleas Nos H 107 R and L, Fig 57 and 58) are interesting in relation to the sensitivity of the inner hair cells One ear showed a complete degeneration of the organ of Corti and associated nerves in the osseous spiral lamina in one sector whereas the other ear showed a loss only of outer hair cells in the corresponding region Still the hearing los wa essentially the same in both ears, 55—70 dB for the frequencies involved (2000—4000 cps) The hearing wa however only tested in octave interval so that it might be possil le that the hearing loss was even more severe for a small frequency band

From animal experiments Lurie (193) suggested that a loss of outer hair cell caused a functional impairment from 30—40 dB Schuknecht (1953 b) con idered that a partial lo s of outer hair cells caused a hearing impairment les than 0 dB; and a total loss about 50 dB The absence of some inner hair cell would cause a los greater than 50 dB Schuknecht mentioned that these relation hips applied to lesions involving frequencies up to 4000 cp wl ereas for higher frequencies the hearing l sses were corresp ndingly more severe

From animal experiment it i known that hearing los caused l y apical lesion i quite different from that produced l y lesions of similar propor tion in the basal end of the cochlea (Walzl and Bordley 1942 Schuknecht and Neff 19 Schuknecht 1953 l) A small lesion n the apical coil may result in no mea ural le hearing los and a larger lesion in a more general los for low tones whereas a basal lesi n t nd to produce a more abrupt and sev re hearing lo Furthermore partial secti n of the au litory nerv clini al and experimental alike point to a difference between the basal and apical region of the cochlea in that the threshold f r higher frequencies i more sev rely affected than that f r the lower (Dandy 1931; Neff 194 ; Wever and Neff 194 Schuknecht and Woellner 19 3 19) It mu t be n ted in thi connection that the audiometric pattern following ul t tal divi ion of the cochlear n rve may be explained at lea t in part l y th spatial di tril ution n the cocl l r n rve of fibers from different part of the cochl a a described by Sando (1965)

Electroph i l g al observation on discharge pattern of ingl u lit ry nerve fibers m l a a hearing on th diff rent ffect of tructural dam g on high and low t es E h of these n rve fibers respon l at threshol l t-

mulus level to a specific frequency (the best frequency or the characteristic frequency of the nerve fiber) For stimuli above threshold, the range of frequencies to which the fiber responds increases (Tasaki, 1954 Katsuk *et al.*, 1958 Kiang *et al.*, 1965) This exp nsion of the tuning curves is more rapid in the low frenquency than in the high frequency units. A further *observation is that fo frequencies below 4000—5000 cps*, the discharges fol l wing pure ton stimulation are spaced at int rvals which are grouped around the integral multiples of the period of the stimulating tone (Kiang *t al.*, 1965 Rose *et al.*, 196) *A strict place theory for the discrim nation* of different frequenc es is not necessary as a princ ple based on time patterns of the respond ng units might provide a mechanism fo pitch discrimination such as that suggested by Wever (1949)

Although parallels might be drawn between the mechanical stimulation pat tern n the cochlea the tun ng *curves* of single auditory un ts, and the present bservations on th relation of hair cell loss to threshold sensitivity for pure tones, a further analysis at present would necessarily be based more on speculation th n on def n tive e dence Crucial nf rmation is lacking on the cru ial l nk between mechanical stimulation of hair cells and dis harge patterns in th auditory nerve especially to the detailed innerva tion pattern in the organ of Corti, and the chemical processes at hair cell — nerve ending level Information n th pathologically altered human cochlea is even more p rse in this respect Observations on cochleas such as in Figs 68 nd 69 in which hearing loss, probably noise-induced f ils to find its correl te n the sensory cell populati n o n th innervation n the osseous spiral lamina emphasizes the need t search further at the level of innerv tion pattern and hair cell — neuronal syn pses fo the nat mico-physiol gic correlates of hearing loss Of course in such cases lesions n the sp ral gang l n nd in high coustic n urons must also be t ken nto consideration

2 D fference limen as revealed by Békésy audiometry

Békésy udi grams w re obtained on s x subjects (1_ cochleas) sh w ng v rious degrees f pure ton he ring loss. In on pai of these cochleas the difference limen was notably diminished, for frequencies ncluding and highe than 3000 cps (Figs 59 nd 60) In regions corresponding to these frequencies a certain loss of both inner nd outer h r cells occured however the loss was not especially severe nd some cochleas w th no change in differ ence limen showed m re pron unced loss of h i cells (Figs. 49 50 53 54)

Often but n t alw ys, a diminished difference limen reflecting n in reased l ud ess sensati n t slight rise n stimulus ntensity at thresh ld level, occurs parallel with the l udness recru tment phenomenon as tested by Fowler s b l nce test Since Dix Hood and H llpike (1948) demonstrated that l udness recruitment was present in Ménière s disease while absent in cases of eighth n rv tum rs, t ha generally been ccepted as strong evidence f r cochlea *dam ge* Th diminished difference l m n as *revealed by Bé-*

kesy audiometry has also been related to cochlear damage Many theories have been propounded to explain the underlying structural correlates of these phenomena Several of these have suggested damage to the hair cell especially the outer hair cells (Tumarkin 1950 and others) however no histopathological investigation has yet convincingly implicated hair cell damage The present study indicates that a considerable loss of hair cells especially outer can occur without a concomitant diminished difference limen as revealed by Békésy audiometry One possible interpretation of this is that the structural correlate is to be sought in the innervation pattern within the organ of Corti

3 *Speech discrimination*

Intelligil ility for undistored speech may be influenced by lesions at all levels of the auditory pathways from cochlea to cerebral cortex, as indicated by Liden (196) Sensorineural deafness due for example, to Meniere s disease and acoustic trauma may be manifested by a discrimination loss, depending on the severity of cochlear involvement Eighth nerve tumors may ause a severe discrimination loss even though the pure tone audiogram shows almost normal threshold According to Neff (1947) Schuknecht and Woellner (1955) and Citron *et al* (1963) an intact residue of some 20 percent of the ganglion cells distributed throughout the cochlea may be sufficient to maintain an almo t normal pure tone threshold sensitivity A considerably larger number of intact ganglion cells is con idered essential to good speech discrimination (Schuknecht and Woellner 19 5; Jerger 1960) Lesion in higher auditory pathways and centers may also cause discrimination deficits Thi i con idered to be the case in subjects showing ph nemic regres ion The speech discriminati n scores are lower than would be expected in view of the pure t ne audiogram (Gaeth 1948)

In the present study six subjects were tested l y speech audiometry Two of them howed normal discrimination a sociated with almost normal pure tone au liograms an l small loss of sensory cell in the cochlea Three subjects (Fig 49 0 53 51 1 5) had speech discrimination scores ranging from 6 percent to 56 percent The pure tone audiograms and the cochlear findings correlated well with these values In one subject the findings in th cochlea differed from what w uld be expected (Figs 59 and 60) Th pure ton au liogram nd th speech discrimination scores (right 9_ percent left 81 percent) were normal for the age (81 years) Tl e sensory cell population were probal ly also within n rmal limit (out r hair cell : bout 1 percent loss with m d rat l a al n l a| e l degeneration inner hair cell : about 3 percent los m inl l al degen ration) The nerve fibers in the osseous spiral lamina h wes r l owed a | rono nced patchy d generation throughout all coil Th overall numbe of nt t n rve fibers wa roughly estimated a about one third of the full comp| m nt In som area tl e degen ration wa almost t tal wherea in oth rs bo t l lf f tl n rve fibers were degen rated Thi finding

is surprising in view of the general opinion that a fairly intact nerve supply is necessary for good speech intelligibility (Schuknecht and Woellner 19 5 Jerger 1960) however it is to be noted that the organ of Corti was fairly intact, with no large area completely denervated, and it may be fair to assume that this llowed the message from all parts of the cochlea to pass without ignificant informational gaps Thus the message reaching the higher centers may have been sufficiently complete for understanding even though the num ber of activated primary neurons was reduced, provided the message were not too complex It seems likely that in the case of a more complex message (e g distorted speech or the like) a functional impairment might become evident. In cases of eighth nerve tumors t is possible that compression affects one part of the nerve more than another resulting in uneven damage with respect to the representation of different parts of the cochlea This being so the message to higher centers then might well become distorted St ll another possibility is that pressure may alter nonuniformly the speed of transmission of nerve impulses and thus modify time patterns of the s gnal

VI GENERAL DISCUSSION

As the sensory region within the labyrinth are enclosed in bone the histological preparation of these structures present special problems. The study of the human cochlea during the last century was carried out to a great extent by aid of micro-dissection techniques and later since the end of the last century these have been replaced almost exclusively by a sectioning technique. This latter technique has yielded a considerable amount of new information about the morphology of the cochlea. The method has since been refined and has contributed further to the knowledge about the normal and the pathologically altered human cochlea. In certain respects, however, exact and reliable information has been impossible to obtain. This is particularly true of quantitative estimates of the sensory cell population and of the innervation pattern within the organ of Corti. The sectioning technique in the evaluation of damage is restrictive in that all parts of the cochlea cannot be studied equally well (Igarashi, Alford and Guilford 196). Decalcification and embedding are processes that are liable to produce artefacts which when present may be difficult to exclude as being representative of the living structure.

In an attempt to study precise quantitative aspects of the sensory cell population and the nerve supply in the osseous spiral lamina the author adopted in part a technique used by Engstrom *et al.* (1962, 1964, 1966) and has further developed other methods for this purpose. These permit an accurate analysis of the cellular pattern in the organ of Corti. Although the technique used does give reliable information as regards innervation density throughout the coil of the cochlea it does not determine the precise number of nerve fibers present. Deviations in overall density are clearly seen and can be accurately related to corresponding regions of the organ of Corti. The method may be extended to include nerve fiber counts in the osseous spiral lamina. A segment of the organ of Corti is removed for examination by the surface specimen technique and nerve fiber counts made on sections of its associated bony lamina following decalcification and embedding. The spiral ganglion can also be studied in a similar way (Kellerhals *et al.*, 1968).

In order to promote further knowledge regarding the normal and the pathologically altered human cochlea a combination of several techniques is necessary. Methods to be used are light, phase contrast and electron microscopy, histochemistry and quantitative chemical analysis. The surface specimen technique is especially suited for studies like the present; however, the technique has the advantage that it does not exclude a combination with other techniques.

Interfering factors such as post mortem changes and artefacts may cause serious errors in the interpretation of the results. The former has been li-

cussed in Chapt IV With experience and care the inclusion of preparation artefacts can be reduced to a minimum Moreover these can under the microscope be clea ly differentiated from pathological changes

It must be considered whether or not the material shows certain characteristics which might influence the findings Almost all of the subjects in the present study had a malignant tumor and were treated at least in periods with nalgenes cytotoxic agents or other drugs A crucial question is then whether or n t these factors to some extent may have caused the degenerative changes described The mention of influence of such factors on human cochleas have been neglected n the literature of recent years As discussed in Chapt. V B the loss of sensory cells before the age of thirty can not be ex cluded to ha e been caused by any of the factors just m ntioned This was indicated by the observations of signs of recent degeneration In the subjects above the age of thirty however no such s gns were observed In animal experiments, the healing of a lesion following sensory cell damage occurs in about two weeks Hence t seems as if the factors menti ned above did not markedly influence the sensory cell popul tion in the weeks before death It cannot, howeve be excluded that toxic nfluence at n earlie period may have caused the more ulnerable sensory cells to degen rat leaving a more resistant population behind Several subjects in the present material have during l fe stated that they had not not ced any sympt m of hearing impairment during the period of disease nd drug treatment A study of cochleas from cases of ccident l death would give aluable nf rmat on on these problems.

The relationsh p of morphol gical changes in the cochlea to presbycusis is difficult to evaluat Such changes might vary from populati n to populat n as ndicated by differencies in hearing uity (Glorig and N on 1960; Rosen t al., 1962) Questions about noise exposure ascul r changes, diet nd genet f tors are closely related t this problem

In material like the present, which ncludes subjects from an urbanized community the quest n about noise exposure is crucial. The cochleas, in clud g those with normal hearing showed decrease in the number of sensory cells with increa ng age The cell l ss was accentu ted at both the basal and ap cal ends of the organ of Corti Cochleas from animals exposed to noise show a different distribution of the sensory cell damage It is not known, however whether o n t the present-day noise in the urbanized environment m y cause degen ration of sensory cells in th cochlea In ord to analyse more closely cochlea changes related to presbycusis, studies ought t be carried out n a large number of cochleas of subjects whose hearing has been tested prior to death. Furthermore subj ts hould be selected from populati ns li ing under different environmental cond t ons.

A group of subjects in the present study were known to hav been exposed to h gh intensity noise f r several years. Som of th cochleas obta ned from these subjects showed lesions n the region f the organ of Cort corresponding to th impaired frequencies (Figs. 5 58 66 67) The changes re in close

agreement with those described in the literature a caused by noise damage (Habermann 1890 Igara hi Schuknecht and M ers 1961) Other cochlea from this group of subjects, however showed no prominent changes in the organ of Corti and in the nerves in the o seous spiral lamina although the audiograms were characteristic for noise exposure (Fig 68 69 72, 73)

Still other noise exposed subjects showed audiograms which were not typical for noise exposure One such example i illustrated in Figs 70 and 71 The outer hair cell population show a circumscribed degeneration in the region one coil from the ba al end (360 21 mm) Circumscribed degeneration at that level of the cochlea have been rarely reported Such lesions were observed in several cochleas of the present study (Figs 18 55 75) Their etiology i obscure

Observations as the above discu sed empha ize that the correlation between morphology function and etiology i very complex Morphological changes at all levels from the sensory epithelium to the central projection areas must be taken into account Also different etiologic factors mu t be consid red a for example heredity Andersson and Wedenberg (1968) observed that sul ject who have a family hi tory of hereditary d afnes may how a narrow dip in the audiogram for frequencies bel w 3 000 cps

VII SUMMARY

The present paper describes the morphology of the human cochlea during its developmental maturation and involutional stages. Particular attention has been paid to the sensory cell population of the organ of Corti nd its nerve supply in the osseous spiral lamina The morphology has been associated with hearing function

These studies have been carried out by a special technique developed by the author used in systematic fashion in the exam nation of cochleas obtained from 29 fetuses, 6 infants and 2 subjects w th age range ng between 1 and 93 years. In 16 of these hearing tests have been carried out

The method entails fixation of the cochlea by perfusion of the perilymphatic spaces via the round window A micro-dissection then follows whereby the bony labyrinth is removed The rgan of Corti and the osseous spiral lamina thus exposed is ex mined macroscop ally as well as m eroscopially Quantitative estim tes made on the sensory cell population are recorded in graphs

In the three-month fetus th cells of the organ of Corti n the whole re undifferentiated except in a narrow one that lies at some distance from the basal end where sensory cells as such can be dentified. With maturati n differentiation proceeds in both basal and pical direction. The fluid sp ces within the organ of Corti begin to develop at the fifth fetal month The ar rangement of the sensory cells is initi lly regular in three and four month old fetuses A gradual ch nge then occur during the fifth and sixth month fol l wing which a slightly irregular pattern forms, s seen in the adult cochle Full d fferentiation of the rgan f Corti occurs at the age of six months

The full complement of sensory cells was estim ted from 10 fetal cochleas The outer h cells show an average number of 13 400 (11,200—16,000) and the inn r a erage 3 400 (2,800—4 400)

The dens ty of sensory cells as measured in numbe of cells *per mm le gth* f the rgan of Corti increases from the basal nd t wards th pex The outer ha r cells almost double their number (290 cells/mm to 525 cells/mm) nd the density of the inne hair cells increases by bout 40 percent (80 cells/mm to 115 cells/mm) The number of out hair cells *pe surface area* is at its max mum at the base and decreases t wards the pex (160 cells/0.01 mm^2 t the base nd 100 cells/0.01 mm^2 t the apex)

With age the total number of sensory cells in the dult cochle shows a clea reduction. Th re are distinct diff rencies between the degenerati n pat t rn in the populati ns of inner nd outer hai cells. The inner hair cells show a degeneration confined to the bas l region of the cochlea, whereas the oute h ir cells sh w an o rall degen rat on which is accentuated at both

its basal and apical end. The nerves in the osseous spiral lamina also show a basal degeneration with age.

In about a third of the cochleas a circumscribed degeneration, patchy at times, occurs independently of the basal and the apical losses present. It consists of either a loss of outer hair cells or a degeneration of sensory cells and nerves in the osseous spiral lamina. The loss of inner hair cells is closely related to the loss of nerves in the osseous spiral lamina. In some cochleas showing these lesions the subject had been exposed to high intensity noise which may well be a cause. In other cases the etiology remains obscure.

A certain loss of sensory cells results in differences in hearing impairment for pure tones depending upon the location of the lesion. Damage at the base where high tones are supposed to be localized gives a considerable threshold impairment, whereas a damage to the apical region of the cochlea can reach considerable degree and still result in a moderate hearing loss.

A diminished difference limen for frequencies of 3 000 cps and higher was found in both ears of a subject examined. This is not related to a structural change associated with a significant loss of either inner or outer hair cells. These specimens show an extensive loss of nerves (about two-thirds of total) throughout the osseous spiral lamina. The subject showed no impaired speech discrimination when tested during life.

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A. EVIATAR and V GOODHILL

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SUPPLEMENTUM [illegible]

From the Department of Surgery, Division of Head and Neck Surgery, Otology Section, UCLA School of Medicine, Los Angeles, California, and the Cedars-Sinai Medical Research Institute, Division of Otolaryngology, Cedars-Sinai Medical Center, Los Angeles

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INTRODUCTION

There is a growing interest in drugs which depress the vestibular system not only with reference to medical therapy for specific vestibular diseases, but also with regard to uses in aviation and space medicine This interest has accelerated the search for new drugs and for new combinations of old drugs.

The effects of drugs on the vestibular system of animals have only recently been studied quantitatively In the past, effects were noted accidentally or evaluated by trial and error methods.

Most of the experimental quantitative methods recently introduced are based primarily on cold caloric stimulation techniques. (The minimal stimulation techniques of bithermal type in clinical use have not proved feasible in animal studies.) There are problems with existing methods for comparative evaluation of the effects of different drugs due to the great individual, as well as species, differences in vestibular sensitivity between animals. In most existing techniques, quantitative evaluations of response decline have been based on comparisons of (*a*) speed of slow component, (*b*) frequency or (*c*) duration of nystagmus.

Classical studies on the mechanism of labyrinthine stimulation by turning of the head or body resulted in several physiological maxims (Mach, Brewer Fleurens, Ewald I and II, and Purkinje–Barany) Barany formalized these conclusions into a pragmatic approach to labyrinthine testing in the Barany rotation test, with use of the Barany chair The torsion swing as a simple rotation test attracted attention mainly because of its simplicity It was described by van Egmond, Groen and Jongkees in 1949 and was traced back to Mach (1875) It is a chair which rotates back and forth within a certain angle. According to the original description, as the initial energy is used up, the speed of rotation decreases and the angle is reduced

The authors propose a method of testing the depressive influence of drugs on the vestibular systems of laboratory animals which employs angular acceleration stimulation and utilizes the principle of sequential comparative studies in the same animal The method will be described and then applied to drug studies conducted in our laboratory

THE CONSTANT ANGLE SWING STIMULATION SEQUENTIAL METHOD

I *The Constant Angle Swing as a Rotatory Test Technique*

Based upon previous studies, de Boer Lorels and Philipszoon (1963) described the use of torsion swing stimulation as a simple rotatory test. Our preliminary experiences with torsion swing stimulation in both animals and humans were also very encouraging and showed advantages over conventional Barany type rotation studies However the variable factors of *time* and *angular decay* inherent in the free torsion effect result in an irregular type of recorded nystagmus with a decaying amplitude and frequency thus making quantitative evaluation difficult This led us to the search for a more controllable method of alternating rotation, which we have found can be achieved by manual force application to the swing.[1] This manual technique permits rotation at a constant angle and constant velocity with the result that a more uniform nystagmus frequency and nystagmus amplitude can be obtained and recorded (Fig 1)

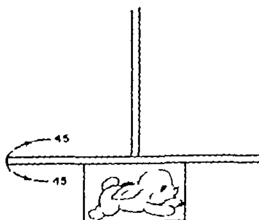


Fig 1 Consta t ngl swing timulation

The time for a 90 excursion in one direction is approximately 2.5 seconds and the speed is maintained for the same time and degree in the *opposite direction The maximal acceleration and maximal velocity values* can be computed by the formula

$$\text{Velocity} \quad \left(\frac{d\theta}{dt}\right)_{max} = \frac{2\pi}{T}\theta_{max}$$

$$\text{Acceleration} \quad \left(\frac{d^2\theta}{dt^2}\right)_{max} = \frac{4\pi^2}{T^2}\theta_{max}$$

A l lt rnating d ice has been designed to replace the manual technique in ou t l es

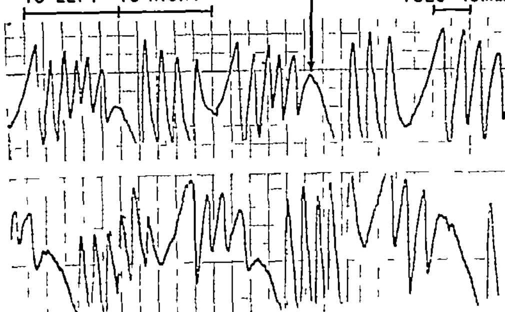


Fig. 2. A nystagmographic sample obtained by constant angle swing stimulation presented here to demonstrate the time and acceleration values.

giving a maximum velocity of 5 degrees/second and a maximum accelera tion of 71 degrees/seconds where the angle $\theta_{max} = 45$ degrees and the period of one oscillation $T = 5.0$ seconds.

To evaluate this technique in animals, it was decided to compare the amplitudes of nystagmus obtained from swing stimulation with those obtained from caloric stimulation techniques. In preparing for the com parative study we were unable to find information relative to the use of alternate cold and warm stimulation in rabbits, nor did we note any studies indicating that rabbits do not respond to warm calorization Investigation of the parameters of bithermal calorization in rabbits was, therefore deemed to be a necessary preliminary step to a comparative study of the two techniques.

Step 1 Comparison of bithermal calorization techniques in rabbits

Healthy rabbits of Dutch strain, weighing 1.5–2.5 kg, were chosen for this experiment due to convenience in handling and suitability for intra venous drug injection techniques. Electronystagmographic tracings in re-

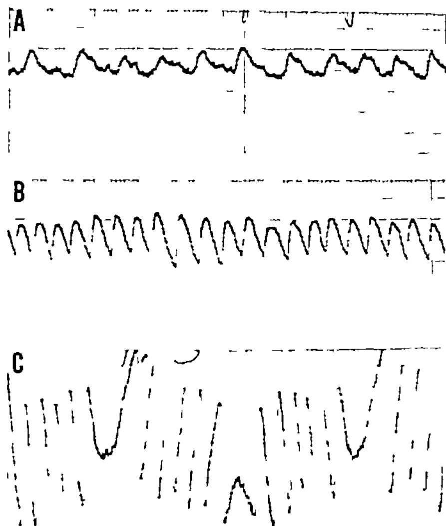


Fig 3 (A) Nyst gmus in rabbit ev ked by cold caloricati n, 10 C, left ear (B) Nystagmus ev k d by cold c l ric ti n, 6°C, l ft ea sam rabbit a i A. (C) Ny t gm i duced by constant angl wi g in n rmal rabbit

sponse to evoked labyrinthine nystagmus were recorded on a Beckman dynograph with rectilinear recording using a sensitivity of 0 1 mv/cm–0.5 mv/cm The electrodes were introduced subcutaneously approximately 4 mm lateral to the temporal canthus of each eye

Drugs were injected intravenously via the auricular vein Effective nystagmogenic doses were determined for each individual animal by trial.

The Beckman Dynograph Recorder used i a two-channel direct writi g rectilinear oscillograph which has maximum sensitivity f 10 microvolts/cm f pen deflection. This heat writing ll transistorized u it is used with the type 9859 Nystagmus Input Coupler The filter has l w-frequ ncy time constant f 5 econds and a high-frequency cut-off t 25 Hz.

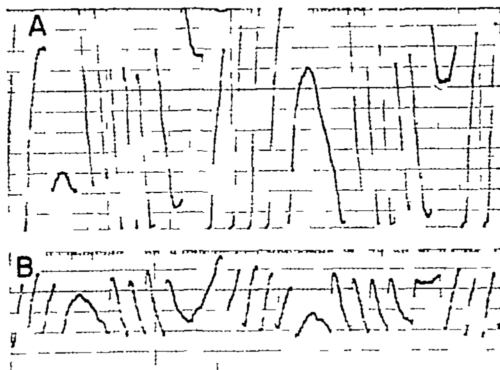
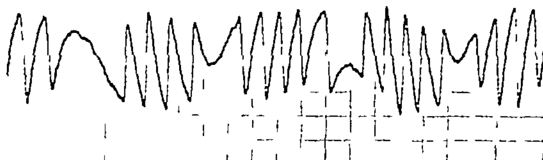


Fig 4 Illustratio of extreme differences in sen itivity of the vestibula ystem i tw normal rabbits. Rabbit A and Rabbit B were recorded by the dynograph in the same sensitivity range The peed f the constant ngle swing wa the same in both recordings. There were no such differences in response in the same rabbit used on different days.

The criterion of dosage choice was adequate suppression (response decline) of induced labyrinthine nystagmus The optimal test dose was considered to be that dose which, upon further increase produced no further depression in induced nystagmus The weight proportion method for dosage calculation on the basis of human therapeutic doses proved to be ineffective in animal studies.

Experiments were conducted in totally dark or semi-dark rooms. Twelve rabbits were used in this study

Results We found that none of the rabbits responded either to cold stimulation by lowering the temperature of the water 7 (to 31 C) or to warm stimulation by increasing the temperature 7 (to 45 C) as is the routine in the Fitzgerald–Hallpike method used in humans. In order to obtain a significant nystagmus response to cold calorization, it was nec essary to lower the temperatures to 10 C (Fig. 3 A) although consistently better recordings were obtained at 5 C (Fig 3 B) We were *unable to elicit* nystagmus by warm stimulation, even with the use of hot water at 60 C for two-minute irrigation periods. The 5 C temperature was thus selected a a tandard temperature for cold calorization in rabbits.



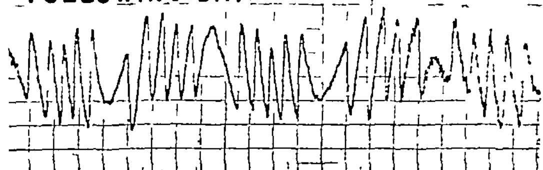


Fig 5 N st gmus recorded i the sam rabbit n different d ys, pri t intrav nous drug i jections. There wa no chang i the amplitude f the evoked nystagmus by constant angle swing stimulation n lternat days in the same rabbit

Step 2 Comparison of constant angle swing stimulation with caloric stimulation in rabbits

The next step was to compare *in the same rabbit* the amplitudes of ny stagmus response to cold calorization at 5 C with amplitudes following constant angle swing stimulation.

Results We found close correlations between the two methods when compared in the same animal, as noted in Figure 3 Fig 3 C illustrates the nystagmus following constant angle swing stimulation in the same rabbit studied by cold calorization in Figs. 3 A and 3 B It will be noted that the constant angle swing responses (Fig 3 C) produced a larger amplitude a feature t lecided value in comparative drug studies. Comparable ampli tude correl t ons were observed in all twelve of the rabbits studied

Bithermal caloric stimulation is used primarily in assessing unilateral lesions of the vestibular system, but there is no need for specifically uni lateral labyrinthine information in drug effect studies, bilateral summation actually being more representative of the total vestibular response to drug actic n We therefore adopted the constant angle swing technique, a rapid simpl meth d o our stimulus approach to quantitative vestibular drug studie

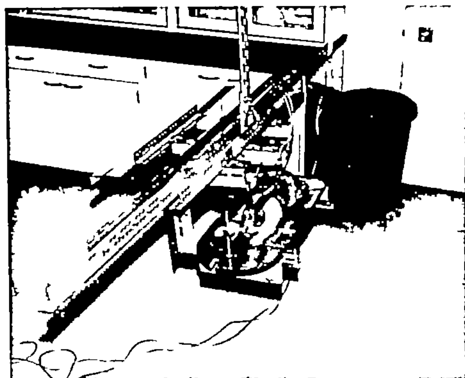


Fig. 4 Rabbit tied i pro position for constant angl swing stim lation

II *Comparative Sequential Drug Studies in the Same Animal*

Having reached the conclusion that the swing method gave us ideal amplitude excursion for assessing the effects of drugs on the vestibular system we then used this method to compare individual differences in sensitivity between two normal rabbits.

In Figure 4 there is a clear illustration of the marked possible dif ferences in swing amplitudes recorded under the same conditions in two different rabbits. Such individual differences were observed in all rabbits tested These individual variations between test animals made it obvious that we could not resort to simple amplitude measurements as a function of drug effect on a group of different rabbits It became necessary to devise a method which would sequentially allow the study of effects of a series of drugs on the same rabbits whose baseline constant velocity angular acceleration swing performance had been established. It had also been found that amplitude response magnitude in the same animal was repeatable to a high degree chronologically (Fig. 5)

These observations led us to the adoption of a method in which the same rabbit would be studied on consecutive days in an attempt to com pare the vestibular suppressive or excitatory effects of a group of drugs. Thus, the animal would serve effectively as its own control in such a drug assay technique

III *The Method*

In order to avoid the problem of individual differences in sensitivity of the vestibular system the effectiveness of different drugs are measured in the same animal each drug on a different day using the same animal as its own control by injection with an equal volume of saline The animal is tied in prone position and checked for spontaneous nystagmus (Fig 6) Then it is rotated on the constant angle (90) swing in a semi-dark room and baseline nystagmus excursions are recorded. After determining by trial the optimal dosage of the test drug it is injected intravenously Rotation by constant angle swing is repeated 10 seconds after the injection and at 15-minute intervals thereafter and nystagmus is recorded

Utilizing this technique two experiments were conducted to test and compare the actions of the drugs on the vestibular system of the rabbits

Conclusions

1 Constant angle swing stimulation is a simple and reliable method of studying the responses of the vestibular system of laboratory animals to drugs.

2. The method provides high amplitude of the recorded nystagmus, more suitable for quantitative evaluation than those obtained by cold calorization

3 It was found that rabbits do not respond to warm calorization and therefore do not lend themselves to reliable stimulation by caloric methods

4 Due to individual differences in the sensitivity of the vestibular system in rabbits, the same animal should be used to compare the effectiveness of different drugs, serving also as its own control

5 No significant differences in individual labyrinthine nystagmus were found between studies performed in completely dark or semi-dark rooms.

DRUG EXPERIMENT I
EFFECTS OF THORAZINE, DRAMAMINE, AND PHENERGAN ON THE FUNCTION OF THE VESTIBULAR SYSTEM OF THE RABBIT

Thorazine is the trade name for chlorpromazine a phenothiazine derivative known also by other names (Largactil, Megaphen, Hibernal) It was developed in France in 1950 and introduced in clinical practice as an aid to anesthesiology one year later Chlorpromazine is known to be mostly a sympathicolytic drug (Courvoiser *et al.*, 1953) centrally depressive especially on the brain stem with pronounced inhibitory effect on the activity of the reticular formation (Hiebel *et al* 1954) Because of this, its effect on the vestibular system was studied by Salerno (1955) Carbonara and Salonna (1956) Bergstrom and Koch (1956) Jongkees and Philipszoon (1960) and others.

Phenergan is the trade name of Promethazine also a phenothiazine derivative known for its antihistaminic action and sedation effect. It was found to be very effective against motion sickness, but is not a drug in common use in vestibular disturbances (Chinn, H I and Smith P K., 1955 Glaser E. M., and McCance P A., 1955)

Dramamine is the trade name for Dimenhydrinate an antihistamine Although it contains 8-chlorotheophylline, the active therapeutic ingredient is diphenhydramine It is used mostly in motion sickness and vestibular disturbances (Gay L. N and Carliner P E., 1949 Campbell, E. H., 1949 Gay L. N., 1951 *a* and 1951 *b*) The mechanism of action of antihistamine drugs on vestibular activity is not completely understood It is presumed to be completely separate from its antihistaminic action and also from its cortical sedative action.

A comparison between these drugs by our method was of interest Dramamine is usually considered a very useful anti-vertigo drug, and its depressive activity on the vestibular system is widely accepted. The effectiveness of Thorazine has been disputed Phenergan had been considered more effective than either Thorazine or Dramamine in motion sickness

Methods

The methods and material used in this experiment were the same as those previously described Ten rabbits of Dutch strain, weighing 1.5–2.5 kg, were used Tied in the prone position each rabbit was checked for spontaneous nystagmus, after which labyrinthine nystagmus was evoked by

constant angle swing stimulation as decribed previously The nystagmus was recorded with the Beckman rectilinear dynograph The drug was injected into the auricular vein Ten seconds after the injection and every 15 minutes thereafter labyrinthine function was induced by constant angle swing and the nystagmus was recorded

Method of Analysis

The nystagmographic comparison was based quantitatively on the average depression of the initial amplitude of the evoked nystagmus as measured before the injection of the drug

Dosages

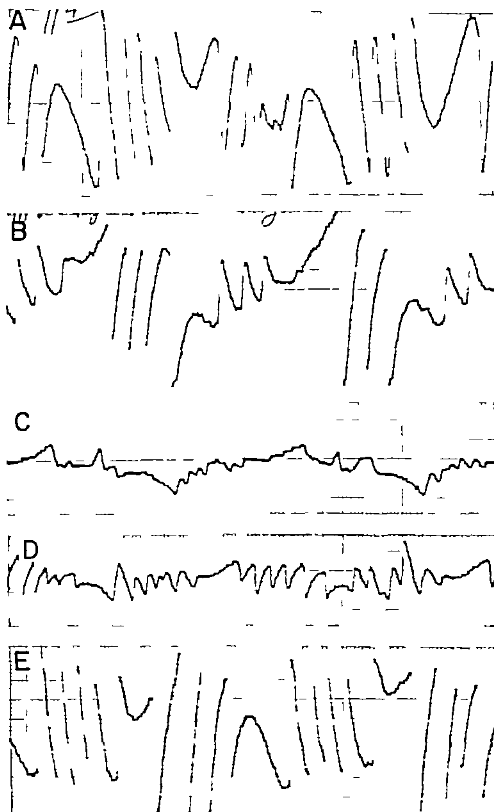
The effective optimal doses of the drugs injected intravenously were determined by trial The selected effective optimal dosages were Thorazine 5 mg/kg Dramamine 10 mg/kg Phenergan 15 mg/kg

The main criterion was that the effective dosage would produce suppression of the induced nystagmus. As previously stated we found that it was impossible to make this dosage determination on the basis of human dosages Thus, it was necessary to adopt a trial technique to work out the most effective dose which would produce reliably repeatable amplitude changes for each drug. The optimal effective dose as selected was such that no further depression in the induced nystagmus could be obtained when dosage was increased even up to sublethal doses. The optimal dose of Phenergan was found to occur very close to the toxic dose (effective dose 15 mg/kg toxic dose, 20 mg/kg) A larger margin of safety was found with Thorazine and with Dramamine (Thorazine effective dose 5 mg/kg toxic dose more than 20 mg/kg Dramamine effective dose 10 mg/kg toxic dose 2o mg/kg)

Results

Thorazine In Figure 7 A shows the baseline nystagmus induced by constant angle swing stimulation before injection of Thorazine B shows a definite depression of nystagmus within 10 seconds after injection of the drug C shows maximum depression of nystagmus after 15 minutes. In D two hours aft r injection recovery is observed as indicated by beginning n reased amplit de of excursions. In E, three hours following the beginning the experim) he normal baseline response to constant angle swing

F (Thorazine inj t d t rabbits, 5 mg/kg, I v (A) Baseline nyst gmus induced by nt ngl wing, b (injection (B) 10 seconds aft t jection, the depress n of (gmus is I iltated ( ) The nystagmu is maximally depressed after 15 min. (D) Aft 2 hours, reco ery is bserved by the mplitudes recorded (E) 3 hours afte the beg ng f the xperiment rmal response t constant ngl swi g stimulation i recorded


A
B
C
D
E

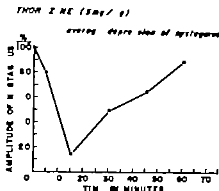


Fig 8. Average depres ion f th mplit de f th I duced nystagmus in the 10 rabbit I jected with Thorazin

stimulation is again recorded and the amplitude is practically identical with that observed in the tracings of A

Thorazine 5 mg/kg i v depressed the amplitude of the induced nystagmus after 15 minutes by an average of 90% (Fig 8)

Dramamine In Figure 9 tracing A shows the baseline of the nystagmus induced by constant angle swing before injection of the drug B shows the initial depression of nystagmus within 10 seconds after injection of the drug C shows maximum depression of the nystagmus after 15 minutes. D shows a recovery almost back to the normal baseline of A after three hours following the injection of the Dramamine

Dramamine, when injected in doses of 10 mg/kg i v produced a depression of the amplitude in the induced nystagmus after 15 minutes by an average of 70% (Fig 10)

Phenergan Tracing A of Figure 11 shows the baseline of the nystagmus amplitude induced by constant angle swing before injection of the drug Tracing B shows beginning depression of the nystagmus after a period of 10 seconds. Tracing C shows maximum depression of the nystagmus 15 minutes following the injection (note the very marked difference between the excursions in tracing C with the other tracings C obtained with Thorazine and Dramamine) D shows return almost to normal response in the amplitude of the evoked nystagmus three hours following the beginning of the experiment The vestibular depressive effect of Phenergan is decidedly the least of these three drugs.

Phenergan, when injected in doses of 15 mg/kg i v., depressed the amplitude of the induced nystagmus after 15 minutes by an average of 60% the lowest of the three drugs studied (Fig 12)

Discussion

Using our new method of evaluation on laboratory animals, the actions of Thorazine, Dramamine and Phenergan on the vestibular system of the rabbit were explored and compared

Fig 9 Dramamine i jected int rabbits, 10 mg/kg. I. (A) Baseline ystagm induced by constant ngl swing before i jecti n. (B) 10 seconds fter i jection, the depression f ystagmus i initiated. (C) The ystagm is maximally depressed fter 15 min. (D) 3 hours fter the begi ing f the xperiment, lmost normal response t const nt gl wi g stim lation i recorded.

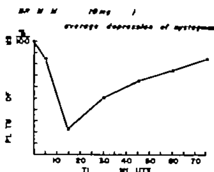


Fig. 10. A erage depression of the mplit de of the induced yst gmus i the same 10 rabb ts injected with Dramamine

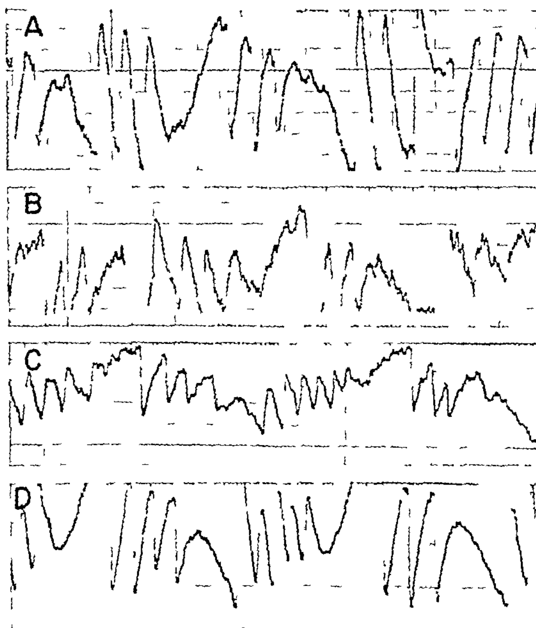


Fig. 11 Phenergan injected int rabbits, 10 mg kg. 1 (A) Baseline ystagmu induced by constant angl swing before injection (B) 10 seconds fter injection the depression f nystagmus) initiated. (C) The n st gmu i maximall depressed fter 15 min. (D) 3 hours fter the beginni g f the periment, almost normal response t const nt angle swi g tim lati is recorded.

Jongkees and Philipszoon (1960) experimenting with rabbits, found that chlorpromazine did not affect vestibular excitability contradictory to the findings of Salerno (1955) Carbonara and Salonna (1956) and Bergström and Koch (1956) Wood (1964) in his review of motion sickness drug literature concluded that chlorpromazine was ineffective in com

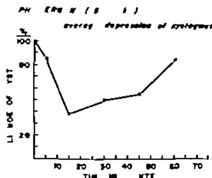


Fig. 12. A erage d pressi f the amplit de f the induced nyst gmus i the same 10 rabbit i jected with Phenerg n.

parison with other phenothiazines and many other drugs. The same conclusion has been found elsewhere (Goodman and Gilman 1966) i.e. that chlorpromazine is ineffective against vestibular disorders and motion sickness It is interesting to mention that Phenergan was stated to be very effective against motion sickness (as would be expected, it being an excellent antihistamine) and the only reason for it not being widely used is its cortical depressive side effect. Dramamine was found to be helpful in motion sickness and vestibular disorders.

In our experiments in rabbits, chlorpromazine was found to be the most active in depressing vestibular nystagmus induced by constant angle swing. Dramamine was only the second in effectiveness, judged by the same criteria and Phenergan was the least active drug

At first glance there appears to be a great discrepancy between our results and those of other authors. These discrepancies can easily be attributed to the different experimental and interpretative methods. A more searching look at the results may suggest an interesting observation chlorpromazine was found by others to be clinically ineffective in motion sickness, with an indirect implied conclusion that it is ineffective as a depressant of labyrinthine function

Our experiments are actually not in contradiction with this concept we found chlorpromazine to be effective against semicircular canal stimulation but we did not study effects on linear motion (i.e. utricular or saccular otolith stimulation) which may be unaffected by this drug There exists also the possibility that Phenergan has a favorable effect in depressing utricular and/or saccular otolith stimulation, while affecting semicircular canal function only slightly Dramamine, an intermediate drug, may act on both canal and otolith systems. The difference in their sites of action may be due to their different chemical formulae Experiments designed to study separation of canal and otolith system responses are underway in our laboratory

Conclusions

In this series of studies to compare the action of Thorazine, Dramamine and Phenergan on the vestibular system of the rabbit

1 Thorazine was found to be the most active drug against semicircular canal stimulation followed by Dramamine Phenergan was the least active drug These results are in reverse order to their clinical activity against motion sickness as reported by others

2 It is suggested that there are differences between drug effects on semi circular canals versus otolith organ function

DRUG EXPERIMENT II
EFFECTS OF NEMBUTAL[1] ON THE VESTIBULAR SYSTEM OF THE RABBIT

Since the discovery of the barbiturate effect by Fisher and Van Mering in 1903, several changes and additions have been made to the malonylurea molecule, with the result that this important group of drugs has become one of the most commonly used.

Barbiturates are widely used to cause a depressive action on the central nervous system. Although it has been established that the cerebral cortex and the reticular activating system are the most sensitive to barbiturate effects, while the cerebellar vestibular and spinal systems are less sensitive (Goodman and Gilman 1966) the barbiturates have, for many years, played a prominent role in the treatment of vestibular disorders and motion sickness (Noble, 1955)

One would expect the barbiturates to depress vestibular activity in subanesthetic doses. Excitatory effects on the vestibular system are suggested by the observation of preanesthetic nystagmus (Bender and O'Brien 1946 Goodman and Gilman, 1960) and nystagmus and ataxia due to chronic intoxication (Hill and Belleville, 1953 Isbell 1951 *Goodman and Gilman* 1966)

The aim of this study was to determine the effects of intraven us Nembutal on the vestibular function of the rabbit

Methods

In this experiment 24 rabbits of Dutch strain, weighing 1.5–2.5 kg, were used Each rabbit was carefully tied in the prone position (Fig 13) to eliminate the possibility of positional nystagmus (Gutman Bergman and Chaimovitz, 1964) and checked for spontaneous nystagmus, after which labyrinthine nystagmus was evoked by constant angle swing stimulation The nystagmus was recorded with the Beckman rectilinear dynograph

Each rabbit served a its own control by a preliminary injection of intravenous saline of the same volume as the Nembutal to be injected subsequently *Ten seconds after the Nembutal injection and then at 15-minute* intervals, the animals were tested for spontaneous nystagmus and then for nystagmus induced by constant angle swing stimulation Recording were again made by dynograph

[1] Generic name—Pentobarbital.

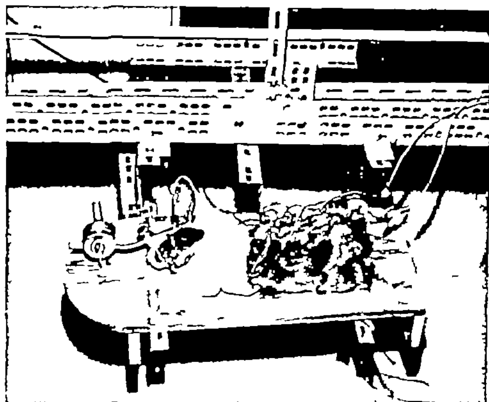


Fig. 13 Rabbit tied in pron position. N posili nal ystagmus can be evoked i this way

(*a*) Six rabbits were injected intravenously each with 7–10 mg/kg Nembutal (about 1/3 anesthetic dose)

(*b*) Six rabbits were injected with 11–15 mg/kg Nembutal (less than 1/2 anesthetic dose)

(*c*) Six rabbits were injected with 20–30 mg/kg (close to a full anesthetic dose)

(*d*) Six additional bilaterally labyrinthectomized rabbits were tested in the same way—two each with 8 mg/kg, two with 10 mg/kg, and two with 25 mg/kg of Nembutal The labyrinthectomy was performed by the ventral approach using the method of de Kleyn and Versteegh The final surgical procedure preceded the studies in all instances by at least 40 days.

Results

(*a*) All six rabbits injected with the small dose (7–10 mg/kg) of Nembutal invariably showed an excitatory effect within 10 seconds on the vestibular system, as judged by the increase in amplitude of the labyrinthine-evoked nystagmus by constant angle swing stimulation. One half hour later n greater amplitude excursions following swing stimulations were recorde F g 14) In one rabbit a short period of spontaneous nystag

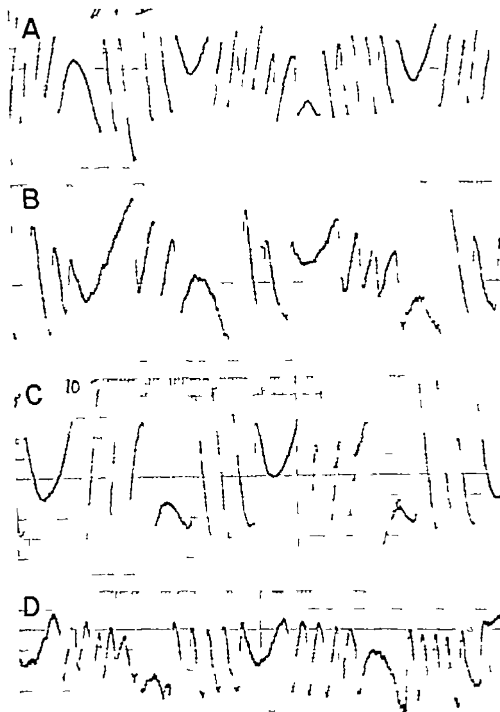


Fig 14 Rabbit injected with Nembutal, 7 10 mg/kg, I. (A) Baselin ystagm respo se t constant ngl swi g bef re i jection. (B) Nystagmus response t constant angl wing tim lati 10 seconds fter i jection The amplitudes re higher tha be-f re the injection. (C) Nystagmus response t consta t gl wing timulati 30 mi fter i jection Not the high amplitudes of the ystagm s. (D) Th recovery 3 hours after the injection, show ystagmus comparable t that in A

mus (10 seconds) was recorded In another rabbit, a short depressive period followed the excitatory period as expressed by a lower amplitude of the evoked nystagmus. In general there was no depressed nystagmus amplitude in this group Excitation was the primary response at this dosage level

(*b*) Of the six rabbits injected with the medium dose of Nembutal (11–15 mg/kg) four showed a preliminary excitatory period expressed by higher amplitude of the evoked nystagmus followed by spontaneous nystagmus (without stimulation) which lasted from 10 seconds to one hour After the spontaneous nystagmus stopped, a depressed period of labyrinthine response with very low amplitudes was recorded (Fig 15) In one rabbit there was no excitatory period, but spontaneous nystagmus was recorded. In one rabbit there was no depressive period following spontaneous nystagmus. Thus, in this group, the outstanding response characteristic was a period of spontaneous nystagmus following initial excitation, followed by final depression

(*c*) The six rabbits injected with the large dose of Nembutal (20–30 mg/kg) invariably exhibited the depressive period Three animals showed only the depressed responses (Fig 16) two had spontaneous nystagmus for a few seconds before the depressive period and one showed excitatory and spontaneous nystagmus phases before the depressive period. This group shows that large doses are followed by depressive nystagmus response which may persist for several hours

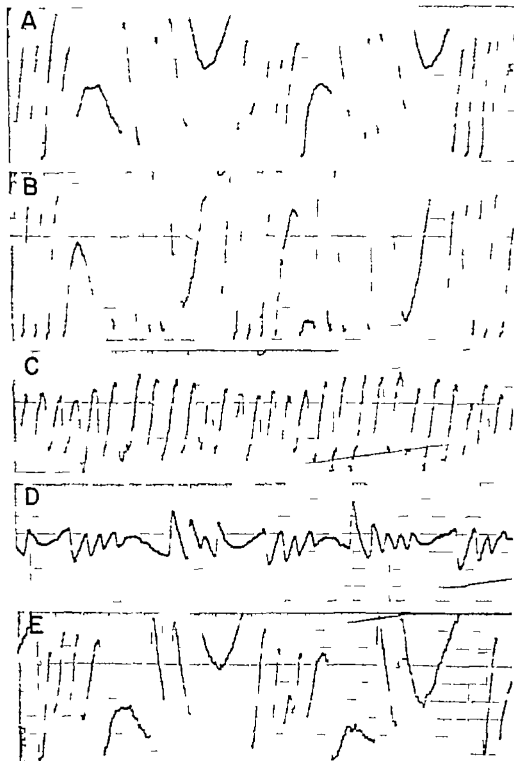
(*d*) The bilaterally labyrinthectomized rabbits showed no nystagmus either spontaneously or following constant angle swing stimulation. Neither intravenous saline nor intravenous Nembutal changed this non responsive state.

In all the control studies, saline injections did not modify evoked nystagmus (Fig 17)

Discussion

Gutman, Bergman and Chaimovitz (1964) in a series of studies on the effect of Nembutal concluded that nystagmus induced in rabbits depends upon head position They postulated, therefore, that the stimulation originated mainly in the vestibular gravity receptors.

Fig 15 Rabbits i jected with N mbutal, 11–15 mg/kg, i.v (A) Baseli nystagmu respo se t consta t ngl wing stimulation bef re th injection. (B) Nystagmus response t constant gl swing stim lation 10 sec after i jecti n. Note the high mplit des f the nyst gmus. (C) Spontaneou nystagmus (with no stim l ti n, f llows the high amplit de period. Thi po ta eo nystagm lasted from 10 sec t on hou (D) The depres i period that ppeared fter th pontaneous nystagmus subsided wa ch racterized by l w amplitud nyst gmus in response t consta t angle swi g timulati (E) Th reco ery period fter 4 hours shows ystagmus response t consta t ngle wi g tim l t n that resembles the nystagmus before injecti


A
B
C
D
E

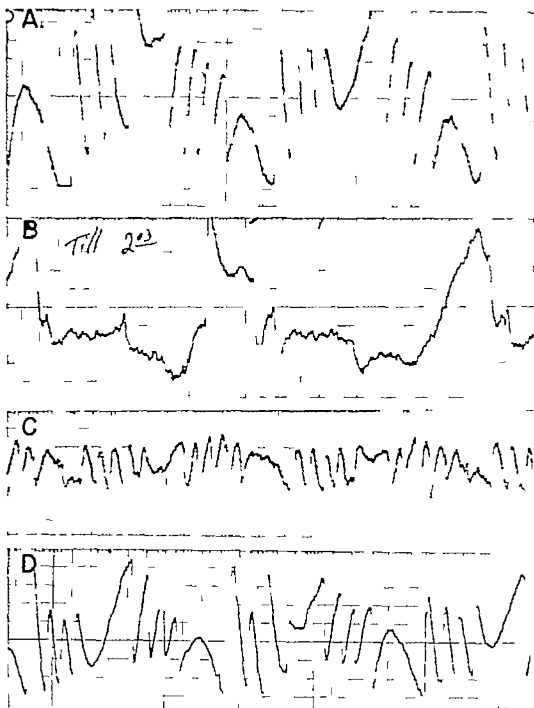


Fig 16. Rabbit injected with Nembutal, 20–30 mg/kg, i. (A) Basell ystagm response t const nt ngl swi g timul ti n before i jection. (B) Th nystagmus response t constant ngl swing stimulati was greatly depressed 10 sec fter i jection. (C) 2 h rs after i jecti n, the nystagmus respo se t con ta t ngl swi g stim lati n recovers, but the amplit des re still smaller than before i jection (D) 4 ho rs fter the begi ning of th xperime t, normal mplit d f ystagm wa recorded, simil to that recorded bef re i jecti n.

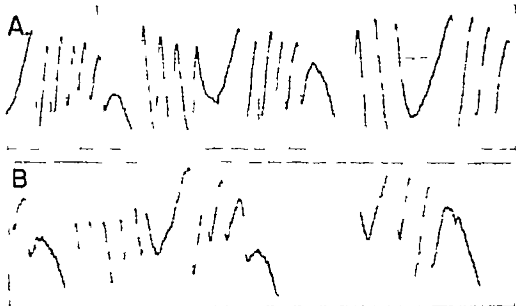


Fig 17 Rabbit i jected with 1 ml salin L served as controls t the experiment. (A) Baseline nystagmus response to constant ngl swing stimulatio before the injec tion. (B) Nystagmus response t constant gle swing stimulation fter injection (10 sec to 2 hours)

Jongkees and Philipszoon (1960) reported that suppression of nystagmus occurs with Nembutal only under very deep anesthesia They did not report either excitatory effects or spontaneous nystagmus.

The route of intravenous administration of the drug and strain dif ferences in the rabbits might account for the differences between our results and the results of others.

In a study on central nystagmus, Oosterveld (1963) showed an enhancement of nystagmus followed by a depression with small doses of Nembutal (2 mg/kg) With larger doses (10 mg/kg) an immediate pronounced long lasting depression of nystagmic responses was obtained Based upon these results and referring to the work of Philipszoon (1959) who came to the conclusion that barbiturates do not affect the vestibular apparatus proper Oosterveld concluded that the mechanism of action of Nembutal is solely central because of depression of central nystagmus only

That central nystagmus also is inhibited by Nembutal is to be expected because of the general central depressive action of barbiturates However our experiments clearly show the direct action of the drug upon the peripheral vestibular complex.

That the effect is mediated via the stato-kinetic end organs was well demonstrated by lack of responses in the labyrinthectomized animal Since our swing produces constant angular acceleration we may assume that

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S U P P L E M E N T U M 238

SIMULTANEOUS MEASUREMENTS OF ENDOLYMPHATIC AND PERILYMPHATIC FLUID PRESSURES BEFORE AND DURING ANAPHYLAXIS AND ASSOCIATED CHANGES IN CEREBROSPINAL FLUID, VENOUS AND ARTERIAL PRESSURES

Candidate Thesis to
the American Laryngological, Rhinological
and Otological Society Inc.
Presented 1965

DANIEL McNAMARA MARTINEZ, M.D

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ACTA OTO-LARYNGOLOGICA

SUPPLEMENTUM [illegible]

From the University of Texas Southwestern Medical School,
Dallas, Texas, U.S.A.

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DANIEL McNAMARA MARTINEZ, M.D.
Assistant Clinical Professor of Otolaryngology

UPPSALA 1968

Supported by N.I.H. Grant No. NB 01617-09

A moving picture in color was prepared, demonstrating the pressure changes t the moment they were produced

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UPPSALA 1969

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INTRODUCTION

The purpose of this paper is to describe a technic for the simultaneous measurements of endolymphatic and perilymphatic fluid pressures in living anesthetized guinea pigs to report measurements obtained under the experimental conditions hereforth outlined in thirty five per cent of over 1300 experiments in a six year study and to describe the effect of anaphylactic shock upon these pressures. The simultaneous measurements of endolymphatic and perilymphatic fluid pressures have not been previously reported

Weille *et al* [38] were the first to obtain and measure individual pressures of the endolymph and perilymph. These were obtained in anesthetized guinea pigs by utilizing microcannulas and capacitance electromanometers. The microcannulas were inserted into either the scala media or vestibuli previous fenestration of the cochlea Perilymphatic pressures were also obtained through the round window membrane Pressure measurements of 14 mm Hg to 32 mm Hg for the perilymph and 8.5 mm Hg for the endolymph were reported Simultaneous pressures were not reported

Perilymphatic and cerebrospinal fluid pressures were obtained simultaneously in the cat by Kerth & Allen [23] The results of 30 measurements on 6 cats were reported. Spinal fluid pressures were obtained by inserting a fine polyethylene tube into the subdural space of the cat after performing laminectomy Perilymphatic fluid pressures were obtained by a capillary tube placed through a cochlear fenestration in the area of the scala tympani Both tubes were connected to water manometers. The authors concluded that the perilymphatic and the cerebrospinal fluid pressures were identical

Other investigators have performed related experiments.

Guild [18] presented experimental evidence that the endolymph flows from the cochlear duct toward the saccus endolymphaticus. He found precipitation of ferric ferrocyanide in the wall of the saccus endolymphaticus after injecting the cochlear duct of guinea pigs with a solution of potassium ferrocyanide and iron ammonium citrate Thus he concluded that there exists normally a circulation of endolymph His theory was that the stria vascularis is structurally capable of forming endolymph which flows toward the saccus endolymphaticus and passes through it into the numerous small blood vessels of this region. Guild compared the stria vascularis to the choroid plexus and secretory areas of the ciliary processes.

Skoog [33] was able to obtain a vestibular syndrome in guinea pigs which was induced by anaphylactic reaction He injected the carotid artery of

living guinea pigs with sheep hemolytic rabbit serum obtaining rotation and lateral flexion of the animal accompanied by nystagmus

Weille *et al* [37] described the effect of anaphylactic shock in the small blood vessels of the spiral ligament and the stria vascularis They noted constriction of arterioles and initial constriction followed by dilatation of the venules. The authors also reported emboli and thrombi in these vessels occurring during anaphylaxis When the animal recovered thrombi persisted

Hallpike & Ledoux [19] determined the osmotic pressures of perilymph endolymph cerebrospinal fluid and blood in the cat They concluded that the osmotic pressure of the cerebrospinal fluid exceeded that of the blood The osmotic pressure of the perilymph exceeded that of the cerebrospinal fluid The endolymph and perilymph were found to be iso-osmotic. They commented that the higher osmotic pressure in the cerebrospinal fluid perilymph and endolymph as compared to that of the blood supports the theory of the origin of these fluids as a secretion the cerebrospinal fluid originating in the choroid plexus and the endolymph and perilymph in the stria vascularis.

Smith *et al* [34] reported that the content of protein was higher in the perilymph than in the endolymph

Naftalin *et al* [33]) suggested that the circulation of the labyrinthine fluids was directed from the perilymph through Reissner s membrane to the endolymph The absorption site was considered to be in the stria vascularis

Becht [9] studied the cerebrospinal fluid pressure in dogs by inserting a trocar into the cisterna magna. He concluded that the venous and cerebrospinal fluid pressures were equal He commented that because of their close physiological relationship it was absolutely necessary to obtain venous pressure measurements when the study of the cerebrospinal fluid was under consideration

Fremont Smith & Forges [16] studying the intra-occular and intracranial pressures, stated that capillary pressure is far more dependent on the venous pressure because of the high resistance to blood flow in the arterioles which are interposed between the arterial pressure and the capillary bed The rate of absorption of fluid in the eye or in the cranium accordingly must vary directly with the venous pressure of the scleral or dural sinuses while the rate of formation will vary with the capillary pressure in the ciliary process and in the choroid plexus Capillary pressure is then primarily controlled by the venous pressure hence provided the osmotic pressure of the plasma remains unchanged the rate both of formation and of absorption must depend on the venous pressure and vary directly with the venous pressure This explains why both aqueous humor and cerebrospinal fluid pressure are so intimately related to the venous pressure and are much less affected by changes in arterial pressure

Hallpike & Cairns [19] studying the temporal bones of individuals who had had clinically Menière s disease and who had died of unassociated fac

tors found gross dilation of the endolymph system affecting chiefly the scala media of the cochlea and the saccule

Lindsay [27] reported the pathological findings of a patient who had severe deafness of sensory neural type accompanied by tinnitus but not vertigo Marked dilatation of the cochlear duct was found He concluded that labyrinthine dropsy constitutes a pathological entity which may produce either auditory disturbances alone or the clinical syndrome known as Menière s disease.

It is considered that there are a number of factors which produce sensory neural deafness and vertigo One of these is an increase of pressure within the inner ear and more specifically in the cochlear duct, resulting in degeneration of the hearing organ. The increased pressure of the endolymph has been explained as occurring secondarily to vascular changes in the stria vascularis. Experimental evidence supports the fact that the endolymph is produced, for the most part, by this structure Allergic reactions or other factors affecting the capillary vessels of the stria vascularis have been considered as a cause for increasing the pressure in the endolymph

The specific aim and objective of this series of experiments is to determine possible changes occurring in the pressure of the endolymph under anaphylactic shock.

I MATERIALS AND METHODS

Over 1300 experiments were performed during a six year study utilizing mostly guinea pigs weighing from 30 to 200 grams. This number also includes 92 cats weighing 2500 to 3500 grams, and 2 rhesus monkeys. Adequate observations were made in 35 per cent of these experiments The remaining were discarded due to flaws in technic incomplete observations, ear disease shock or death of the animal during the experiment Two human perilymphatic pressures were obtained at the time of labyrinthectomy surgery

The guinea pig was chosen as the experimental animal because of its relatively small size and because its cochlea can be made surgically accessible for this type of experiment The cat was utilized in other experiments in order to obtain a comparison with the results obtained in the guinea pig. The rhesus monkey was utilized on two occasions to adapt the procedures developed in lower mammals to the study in primates.

Guinea pigs, free from disease were sensitized with egg white intraperitoneally No animal was presented with a shocking dose until 21 days following the initial sensitizing dose Each animal was anesthetized by the intra-peritoneal injection of sodium pentobarbital A tracheotomy was performed for the administration of oxygen and the cochlea was surgically exposed Two fenestrae were made under microscopic visualization of the cochlea one in an area corresponding to the scala media and the other over the scala tympani Care was taken not to penetrate into these scalae in order to avoid fluid leakage Two quartz micropipettes measuring 30 to 50 micra outside diameter were then driven through the microfenestrae with a micromanipulator thus breaking through the minimal thickness left in the endosteal layer The scala media and the scala tympani were entered in this manner The micropipettes sealed the bony walls as they penetrated and leakage was avoided When leaks did not occur suction in the bulla cavity did not make any variation in our monitoring oscilloscope The micropipettes were attached to their respective transducers by means of a plastic tubing The transducers were attached to suitable pre-amplifiers. The output was monitored by an oscilloscopic screen or two voltmeters and recorded on tape or photographic film. Calibration of the equipment was done before and after the experiment. After the pressures were noted the animal was placed in anaphylactic shock by the intravenous or intra arterial injection of egg white Observations of the changes during anaphylaxis were then obtained and recorded One can determine the position of the



Fig. 1 Microdrill and cochlea. G Inea pig

pipettes by studying the tracings obtained at the time of the experiment. Each pressure had its own characteristic responses

A *Equipment*

1 *Table* In order to eliminate as many vibrations as possible the experimental table was constructed according to the method of Peek & Hoerr The table consists of a thick slab of boiler plate measuring 122 cm square and 1 cm in thickness, the four corners of which rest on four pyramids of squash balls held together in steel cups, and a heavy steel table with an adjustable top on which the cups rest and rubber shock absorbers which support the heavy steel table

2 *Micropipettes* These pipettes were constructed of quartz tubing measuring 7 mm o.d and o.2 mm i.d which had been heated and drawn so that the point would have an outside diameter of 30 to 50 micra. The pipette then tapers outward to minimize capillary pressure The total length of the pipette was .5 cm

Recorders The output voltage of the pre-amplifiers was fed in some instances, into a cathode-ray oscillograph recorder In other instances it was directed into an Ampex (Model Sp-300) Instrumentation Recorder

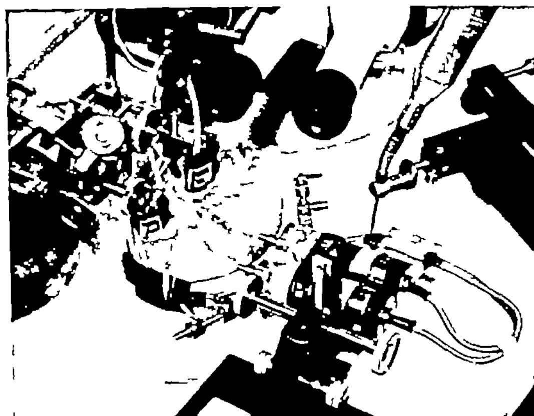


Fig 2. Apparat used in btaining im it eou pressures.

B *Technic*

1 *Sensiti ation* Each animal was sensitized by two 0.5 ml intraperitoneal injections of 1 10 solution of crude sterile egg white five days apart No animal was presented with shocking dose until 21 days or more after the initial dose No attempts were made to quantitate the degree of anaphylactic shock.

2 *Anesthesia* Initially each animal was anesthetized by injecting intra peritoneally 45 mg sodium pentobarbital per kilogram of body weight Further doses are administered as required during the course of the ex periment

3 *Surgical procedure* The operative procedure is divided into four parts (a) the macroscopic exposure of the cochlea (b) the tracheotomy (c) the microscopic fenestration of the second or third cochlear turn over the scala media and the first turn over the scala tympani, and (d) the introduction of the pipettes.

The animal hair around the head and neck regions was removed with an electric razor in preparation for surgery The macroscopic exposure of the cochlea began with a postauricular incision extending into the neck and following the posterior border of the ramus of the mandible The post auricular muscles were separated from their pinnal attachments, the ex

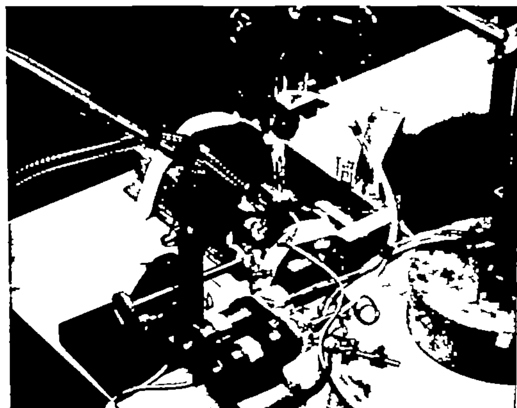


Fig. 3. Experiment in progres obtaining imultaneous endolymphatic nd perilymphatic pressure

ternal auditory canal was transected and the anterior auricular muscles were severed The pinna and external auditory canal were retracted superiorly and anteriorly exposing the bulla The periosteum of this structure was elevated along with the soft tissues surrounding it. The lateral wall of the bulla was then removed exposing the cochlea The stapes was left un disturbed In other instances, the aproach was through a submandibular incision The bulla was dissected and an opening was made in it to expose the cochlea.

In order to dispense with respiratory movements which interfere with microscopic manipulations and observations, a tracheotomy was performed Following the technic of Irwin, a tracheal cannula which only filled two-thirds of the tracheal lumen was inserted. When oxygen under sufficient pressure was delivered through the cannula, respiratory movements ceased The gross surgery was completed and the guinea pig was placed on the experimental table so that the fenestrations of the cochlea could be made with the aid of a microscope

Under microscopic visualization, attention was placed on an area limited by the two bone partitions that separate a cochlear turn where the pigment of the tria vascularis shows in dark animals. The mucous membrane over

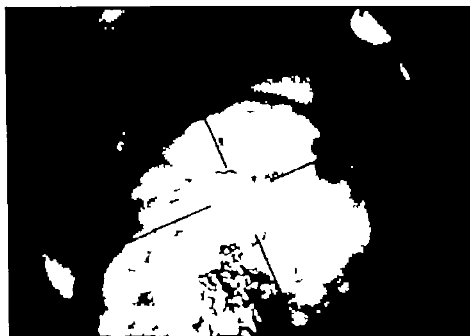


Fig 4 Micropipett in scala media, G inea pig

lying this area is removed with a sharp pick Using the Shea microdrill (Fig 1) with pointed burr and supported and directed by a micromanipulator the bone overlying the scala media of the third turn was thinned down to its endosteum A similar procedure was done on the first cochlear turn immediately above the border of the round window niche in the area of the scala tympani Care was taken to avoid drilling over the veins in the substance of the bone The crater of the fenestra measured 20 to 50 micra The facial or jugular vein was then exposed A cannula containing mammalian Ringer s solution and Heparin was inserted into the lumen of the vein In other experiments the common carotid artery was cannulated for intra arterial injections The cannula was attached to a syringe containing the shocking dose of egg albumin To penetrate the cochlea, two specially designed micropipettes were required These were supported by two micromanipulators which in turn are on their respective stands In this manner the vibrations incurred during adjustment of one pipette were not transmitted through the system to the other pipette The micropipettes were then filled with mammalian Ringer s solution and connected by means of plastic tubing to appropriate transducers to convert the mechanical pressures to an electrical signal for recording Attention was directed to avoid any air bubbles in the system The transducers were placed level with the cochlea by mean of their micro-manipulators and opened to atmosphere for balancing (Figs. 2 3)

Once the system was balanced the quartz micropipettes were then driven through the micro fenestrae by means of the micromanipulators thus break



Fig. 5 G inea pig cochlea micropipettes i place bt l l g lm lt con endolymphatic nd perilymphatic pressures.

ing through the minimal thickness left in the endosteal layer of the scala tympani and the scala media. The tapering portion of the pipette sealed the walls of the fenestra without leakage (Figs. 4 5)

The initial pressures having been recorded, anaphylactic shock was produced in the animal by administering an intravenous dose containing 0.5 ml of egg white In other animals, anaphylactic shock is produced by the intracarotid injection of egg white Simultaneous recordings of endolymphatic and perilymphatic pressures were continued during this last phase of the procedure.

Simultaneous measurements of cerebrospinal fluid and perilymphatic pressures were also performed in guinea pigs and cats

In the guinea pig, no source of information was found regarding the approach to obtain these pressures It was not possible to obtain cerebrospinal pressures by lumbar puncture Dissection of the lumbar region showed that the cauda equina was compressed into a small place with no space available for needle puncture. Initial attempts at cisternal puncture resulted invariably in the death of the animal due to the destruction of the pneumotaxic or respiratory center in the medulla A method was then developed of surgical exposure of the cisterna which was followed by

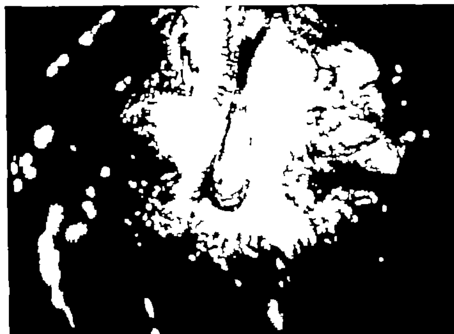


Fig. 6 Polyethylene catheter in cisterna magna. Guinea pig.

introduction of polyethylene tubing into this area. The surgical procedure consisted of making a vertical incision in the skin of the nuchal region of the guinea pig dividing the semispinalis capitis and rectus capitus muscles in order to expose the atlantooccipital ligament. This ligament was then punctured and a polyethylene tubing inserted (Fig 6)

An alternate approach to the cerebrospinal pressures of the guinea pig consisted in placing a short 20 gauge needle into the lateral ventricle of the brain through a craniotomy approach (Fig 7)

In a number of dissections of the guinea pig brain it was noted that the lateral ventricles, which were rather flattened dorsoventrad, were relatively extensive in antero-posterior and mediolateral dimensions. Therefore a craniotomy was performed through the parietal bone of the skull along the point of attachment on the temporalis muscle and about 5 mm lateral to the sagittal suture The skull was exposed by an L shaped incision of the scalp subcutaneous tissues and periosteum extending from the midline between the eyes to the superior nuchal line and then laterally to the auricle The temporalis muscle was detached at its insertion A trephine measuring 3 mm in diameter was made and the dura was exposed A small opening was then burned in the dura utilizing an electrocautery needle Pressures were obtained by means of a needle prepared specially for this purpose A 2.5 cm 20 gauge hyperdermic needle was modified by plugging the tip of the needle while retaining a sharp point Two openings slightly less than 1 mm in diameter were made in both sides of the shaft about 2 mm from the tip This was designed to avoid obstruction of the needle by cortical



Fig 7 Methylene bl dy indicates place f entrance of the pipett in the lateral ventricle and i the istern magna

tissue during its insertion The needle was then attached directly to the pressure transducer In previous experiments, the position of the needle in the lateral ventricle had been established by the injection of 0 1 ml of methylene blue dye through a side arm between the needle and the trans ducer After withdrawal of the needle the brain was removed and sectioned.

The surgical approach for cerebrospinal fluid pressure studies in the cat consisted of a laminectomy in the lumbar region. The dural sac was ex posed and intubated with a polyethylene catheter through a small opening in the dura. This procedure proved to be self-sealing when administered correctly (Fig. 8)

In order to obtain perilymphatic fluid pressures in the cat, a 20 gauge needle with the bevel removed was inserted into the scala tympani through a cochlear fenestration (Fig 9) The surgical approach consisted of a submandibular incision exposing the tympanic bulla, portions of which were

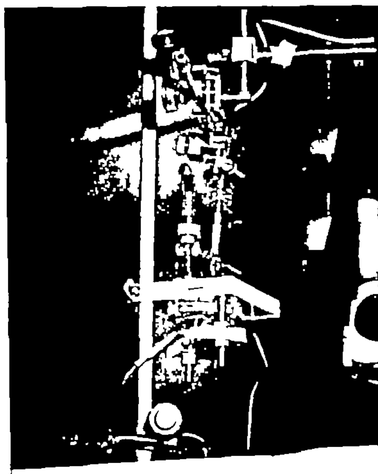


Fig 10 Pressure imul tor for calibrati f hydraulic yst m

of the aforementioned functions. A device was constructed so that pressures of various wave forms and repetition rates could be produced and applied simultaneously to a transducer through a minimum of floistance [1] and to the second transducer coupled through a catheter for the second test

The pipette was constructed of quartz pyrex glass measuring 7 mm o d and 5.2 mm i d The over all length of this pipette was 7.5 cm and the di tal end has been drawn to a tip measuring under 200 micra in length and 30 to 50 micra o.d The catheter used is of the polyethylene type measuring 5 mm o.d and 3 mm i.d The length of the catheter was originally 65 cm This length was shortened to 20 cm in a later experiment Figure 10 h ws the pressure simulator which was fabricated of laminated plastic sheets into which a cavity (4.3 cm in diameter 1.25 cm in depth) had been drilled A corrugated diaphragm was placed over one opening and was

[1] Fl ist nce i defi ed the tot l ppo ition offered t the pa sage f fl id gas. Thi terminology i sed in pl ce f "resist nce which generally designates pposition t the passage of n lectro current. Floi t nce i sed t id confusion nd t fford ca re dability

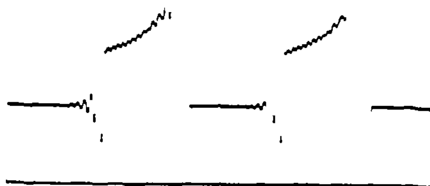


Fig 11 Transducer co pled directly t pressure imulator

held in place by a plastic ring An electromagnet is used to apply force to the diaphragm Maintaining the same magnitude of current supplied to the electromagnet at various frequencies insured that the same force would be applied to the diaphragm

Figure 11 shows the wave form which was produced by this device and recorded on a photosensitive paper The light source was supplied by a cathode ray tube The rise time of the pulse was 0 01 seconds, duration time 2 0 seconds and decay time 0 01 seconds. The repetition of this pulse rate was 3 0 per second It will be observed that the square wave was not completely flat the slight tilt was the result of a relatively high internal impedance of the power supply energizing the electromagnet.

This figure shows the wave form recorded at a repetition rate of 3.0 per second The transducer used in this experiment was Statham Model P 23D and Statham Model P 23BB. These transducers were suitable for monitoring all pressures that were encountered in physiological experiments.

In the following experiment two transducers (Statham P 23BB) were used. Transducer No 1 was connected directly to the pressure simulator through a minimum of floistance (Fig. 11) transducer No. 2 was coupled to the pressure simulator through a 65 cm catheter previously mentioned (Fig 12)

Figure 12 shows the effect of the hydraulic dampening When coupling mediums such as catheters with high floistance characteristics are being employed, it becomes necessary for correction factors to be used in order to obtain correct pressure readings.

Fig 12. Tra sd cer coupled t pres u imulator through l g pla tic tubing.

Fig 13 Tra sducer coupled t pres ure imulat through sh rt pl stic t bi g.

Figure 13 shows a simultaneous pressure reading where one of the transducers was coupled into the pressure simulator through the previously mentioned pipette Parameters, which determine the correction factors where flolstance was experienced in transducer devices, were the displacement factors of the strain gage and the natural resonant frequency of the transducers

Determining a procedure for measuring a displacement factor for the Statham Pressure Transducer Type I 23D was as follows

A 0–5 Lambda pipette was affixed to a standard two-way Luer type stop cock, exerci ing care to avoid leakage The assembly thus described was then attached to the I 23BB strain gage and the system filled with boiled distilled water[2] Precaution was taken to eliminate air penetration in the system Placed al ngside the pipette was a steel rule calibrated in milli meters. The spacing of the calibration of the Lambda pipette was 14 mm per Lambda. Pressure was then applied to the closed system from the open

Di tilled w ter w boiled t remov ny ext ll g al bubbles.

t la nlo-largng 5 ppl 231

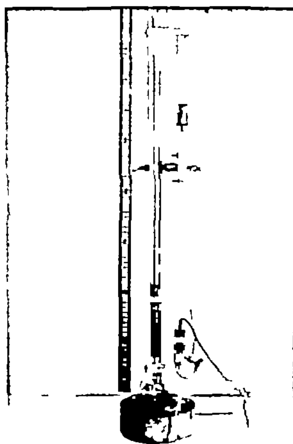


Fig 14 W ter man meter for calibrati f tire syst m.

end of the pipette by use of a standard sphygmomanometer pressure bulb This pressure was also applied by means of a Y tube to a precision differential mercury manometer

The following information was obtained statistically from ten consecutive determinations

Applied pressures (mm. Hg.)	Displacement column (vg.)	
10	0.1 lambda	(One lambda is
20	0.32	equal to 1/1000
40	1.0	of ml.)
60	1.35	
80	1.64	
100	2.1	
150	2 98	

It was necessary to employ transducers which require a minimum displacement because of the limited amount of fluid in the scala media The

fact that inner ear pressures were obtained signified that the displacement factor was less than the amount of fluid pressure present

It was obvious that the displacement factor of the strain gauge was not linear over a wide dynamic range of pressures however under a small operating range of 0 to 10 mm of mercury pressure, it can be assumed that the displacement factor of this strain gauge was essentially linear Attempts to measure the displacement at lower pressures was deemed impractical due to the minute movement of the fluid in the pipette

The resonant frequency of the transducer may be calculated from Frank s equation

where F = frequency in cycles M = mass, D = displacement

$$F = \frac{1}{2\pi \times \sqrt{M/D}}$$

(*b*) Calibration of th entire hydraulic pre-amplifier and recording system (Fig 14)

This wa done with known static loads. A water manometer was made using a column of glass tubing approximately one meter in length This was mounted n a plexiglass board in a vertical position with a stop cock at the lower end A meter stick was mounted immediately adjacent to the glass column and through a polyethylene tubing, the bottom of the column was c nnect d t the fluid chamber of the transducer The transducer was then adjusted s the middle of the diaphragm was at the zero mark on the meter stick, and was level with the meniscus of the fluid in the manometer At thi point the system wa at atmospheric pressure and the pre-amplifiers were balanced and adjusted with a marker on the baseline A fluid reservoir c ntaining boiled distilled water was connected through the stop cock to the fluid chamber f the transducer Water was then allowed to flow from the reserv ir of th fluid chamber into tubing connecting the transducer with the man meter and then into the manometer burette raising the level in increments of 0.5 mm Hg As each level was reached the flow was stopped and the pressure recorded n tape It was then equivalent to that height of water which could then be c nverted into millimeters of mercury With thi meth d calibration was obtained from atmospheric pressure to 10 mm Hg in increments of 0.5 mm Hg After the highest level was reached water wa allowed to fl w out of the system in increments of 0 5 mm Hg until the fluid level in the manometer wa again at the center level of the transducer s diaphragm At this time the system was again at atmospheric pres ure and the marker on the oscilloscope was on the baseline When the phot graphic film registering these changes was devel ped each segment depicted the distance from the baseline w n nded with the actual pres ure in millimeter of mercury or ntin t ater exerted n the transducer diaphragm When the p n a curve the transducer were f und to sh w a ten millimeters f mercury

5 *Difficulties encountered during the development [illegible] obtaining simultaneous pressures*

(*a*) Anesthesia since the amount required for adeq[illegible] very close to the lethal dose

(*b*) Surgical trauma during exposure of the cochlea

(*c*) Fracture of the cochlea during fenestration or durin[illegible] of the pipette

(*d*) Minute particles of bone or tissue obstructing the [illegible]

(*e*) Separation of the spiral ligament from the bone wall [illegible] due to a dull pipette. Negative pressures were obtained

(*f*) Fluid leaks caused by injury to the spiral ligament d[illegible] tion.

(*g*) Leaks due to lack of perfect seal between pipette and [illegible]

(*h*) Capillary bleeding during penetration of the pipette

(*i*) Leaks around the stop cocks and around the attachme[illegible] pipette and transducer

(*j*) Air in the system

(*k*) Capillary action between a moist cochlea and the [illegible] during penetration causing the system to lose its balance

(*l*) Inadequate balancing due to cochlea and transducers [illegible] ferent levels.

(*m*) Excessive lengths of tubing utilized for connection[illegible] system

(*n*) Inadequate sensitization of the animals preventing th[illegible] of anaphylactic shock

(*o*) Cerebrospinal fluid leak from the subarachnoid [illegible] needle.

(*p*) Electronic problems such as calibration, rec[illegible] ference

es
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ing,
rial

II OBSERVATIONS

In order to comprehend the general physiological action of anaphylaxis in the inner ear fluids, it was necessary to perform experiments in which arterial venous, and cerebrospinal fluid pressures could be obtained Data thus gathered would provide a basis for the understanding of how these pressure would affect the endolymphatic and perilymphatic fluid pressures.

The degree of anaphylaxis in the guinea pig of average weight varied however as a general rule it can be said that the maximum reaction to the shocking dose was obtained on the 25th day from the initial sensitization dose The animal that had the greater amount of anaphylactic shock registered the higher peaks of arterial pressure venous pressure cerebrospinal fluid pressure and endolymphatic and perilymphatic pressures Decreased reaction was obtained when the shocking dose was given intra arterially as compared to the intravenous administration (Figs 31 34) There appeared to be no difference in reactions whether the animals were either albino or colored Controlled studies were done in which a similar amount of Ringer s solution was given intravenously to sensitized animals, and egg white given intravenously to non sensitized animals In either case the pressures did not change

A Simultaneous endolymphatic and venous pressures before and during anaphylaxis in the guinea pig (Figs. 15 16)

The average initial or pre-shock endolymphatic pressure in the guinea pig was 2 mm Hg The normal variations in satisfactory experiments were from 1.3 mm Hg to 3.2 mm Hg.

The average endolymphatic fluid pressure during anaphylaxis was 4 0 mm Hg with variations between 2.2 to 5 8 mm Hg

The average initial or pre shock venous pressure was 3.5 mm Hg with variations from 1.25 to 8 0 mm Hg.

The average venous pressure during anaphylaxis was 9 0 mm Hg with variations from 5 mm Hg to 14 mm Hg.

The highest endolymphatic pressure peak during anaphylaxis occurred on the average at 6 minutes and 20 seconds after the shocking dose The greatest increase in pres ure ocurring within 2 minutes

The highest venous pressure peak during anaphylaxis occurred on the average at one minute and 25 seconds after the shocking dose

The experiments howed con i tently that the venous pressure was higher than the endolymphatic pres ure Endolymphatic and venous pressure

were noted to start their rise at an average less than 14 seconds after the injection of the shocking dose However the venous pressure peak appeared earlier than that of the endolymphatic peak The duration of the endolymphatic and the venous pressure rise during anaphylaxis was quite variable On the average, both pressures returned to their pre-shock level from three to four minutes after the injection of the shocking dose When the animal survived, the venous pressure returned to its pre-shock level however the endolymphatic pressure remained always slightly higher than its pre shock level During the death of the animal, the venous and endolymphatic pressures equalized and continued decreasing together until they reached the baseline The endolymphatic pressure remained positive for an average of 60 seconds after the venous pressure reached the baseline

B *Simultaneous cerebrospinal and arterial pressures before and during anaphylaxis in the guinea pig*
(Figs. 17 18, 19 20 21 22 23)

The average pre-shock cerebrospinal fluid pressure was found to be 4.5 mm Hg obtained both in the cisterna magna and in the lateral ventricles of the brain

The average or pre-shock arterial pressure measurement obtained in the carotid artery was 28 6 mm Hg

Maximum pressure of the cerebrospinal fluid during anaphylaxis was 9 0 mm Hg at the first peak and 8 6 mm Hg at the second peak

Maximum carotid arterial pressure during anaphylaxis was 47.8 mm Hg Time of occurrence of the peaks from the beginning of the shocking dose were as follows

Cerebrospinal fluid pressure first peak, 1 minute and 50 seconds.

Cerebrospinal fluid pressure second peak 8 minutes and 5 seconds.

Arterial pressure peak, 1 minute and 24 seconds from the shocking dose

Immediately after the shocking dose was administered to the animal, either intravenously or intra-arterially the cerebrospinal fluid and arterial pressures began to rise. The arterial pressure rise was more sudden. The cerebospinal fluid reached its peak about 26 seconds after the arterial pressure peak had been attained.

In 50 per cent of the experiments, the cerebrospinal fluid and arterial pressures slowly returned to their preshock level In the remaining 50 per cent of the experiments, the cerebrospinal fluid pressure remained at a level slightly higher than the pre-shock level (5 7 mm Hg average)

While the arterial pressure continued to fall to the initial level, the cerebrospinal fluid pressure rose again to an average pressure of 8 6 mm Hg The time of occurrence of the second cerebrospinal fluid peak was 8 minutes and 5 seconds from the injection of the shocking dose This second rise of cerebrospinal fluid pressure while the arterial pressure was decreasing, was suspected to be secondary to cerebral edema since there was no arterial pressure rise to explain this second cerebrospinal peak

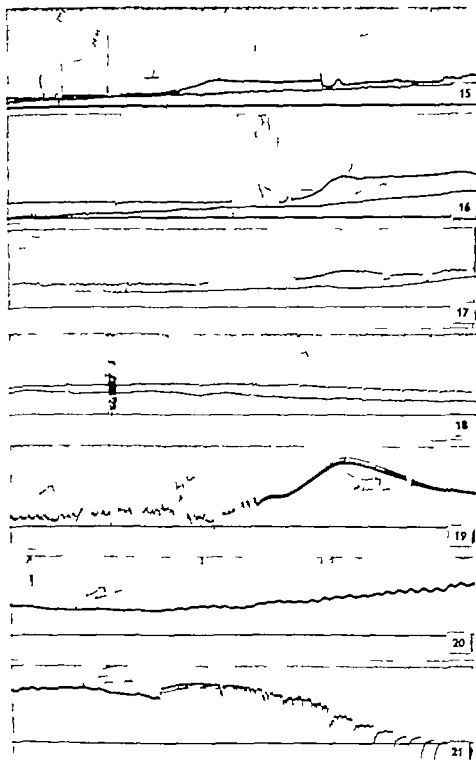


Fig. 15. Sim lta eous endolymphatic nd venous pressures duri g anaphylaxis. Guinea pig. Endolymph lower traci g. Not f ater rise i venous pressure

Fig. 16. Simultaneous end lymphatic nd nous pressures d ri g n phylaxis.

Fig. 17 Simulta eous CSF and carotid pressures during naphyl is.

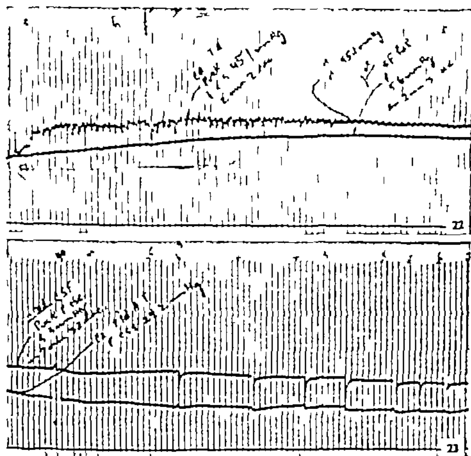


Fig 22. First CSF peak d ring naphyl xis

Fig. 23. Same periment. Seco d CSF peak arterial pressure decreasi g.

In other experiments, the ligations of one or both internal jugular veins was performed. When one vein was ligated 1 mm Hg rise was obtained in the cerebrospinal fluid pressure as compared with the pre-ligation record ing. When both internal jugular veins were ligated there was an increase of 2 mm Hg from the pre-ligation level and the rise in the cerebrospinal fluid pressure was sustained. Neither the cerebrospinal, nor the arterial pressure proximal to the brain changed appreciably when one carotid artery was ligated.

It is to be noted in the illustrations submitted that the tracings have dif

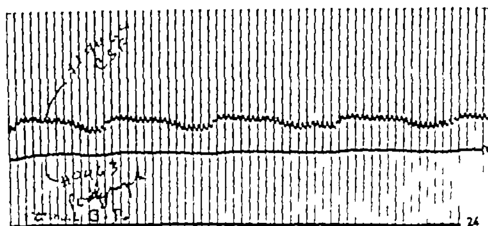
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Fig. 18. Same experime t. Second CSF peak during anaphyl i ; arteri l pressure decreasing.

Fig 19 Cerebrospinal fluid pressure d ring naphylaxis. G inea pig.

Fig. 20 S n experiment. F ll f CSF pressu after first anaphylactic rise

Fig. 21 Same xperiment. Second rise of CSF pressure probably due t cerebral edema.



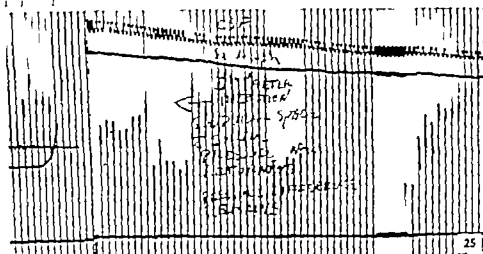


Fig 24 Simult neous perilymph nd CSF pressures Guinea pig

Fig 25. Simulta eou perilymphatic nd CSF pressures. Cochlea duct pat nt. Injecti n of 1 ml Ri ger's solution i to subdural space Cat.

ferent calibration factors for measurements, since the different pressures studied have different ranges.

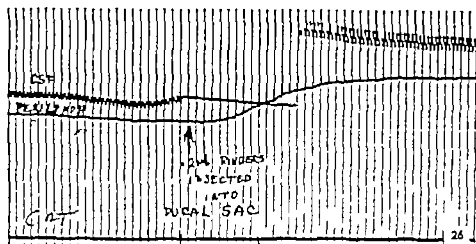
Only extreme positions of the animal s head varied the cerebrospinal fluid pressures obtained In other experiments the animal was placed in the vertical position. Positive cerebrospinal pressures were still obtainable

C. Simultaneous perilymphatic and cerebrospinal fluid pressures in the guinea pig before and during anaphylaxis (Fig 24)

The average pre-shock perilymphatic fluid pressure was found to be 3.5 mm Hg

The average pre-shock cerebrospinal fluid pressure was found to be 4.5 mm Hg

The maximum perilymphatic pressure during anaphylaxis was found to be 6.2 mm Hg. A second peak was not noted in most cases



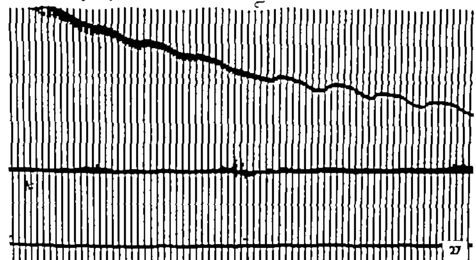


Fig 26 Sam type f experiment. H wever I jecti f 0.2 ml Ri ger's solution int subdural pace.

Fig. 27 Simultaneou perilymphatic nd CSF pressures. Cochlear duct occl ded. I jec tion f 1 ml Ringer' sol tio int subdural pace Cat.

The average maximum cerebrospinal fluid pressure during anaphylaxis was 9.0 mm Hg at the first peak and 8 6 mm Hg at the second peak

The first cerebrospinal pressure peak occurred 1 minute and 50 seconds after the shocking dose, and the second peak at 8 minutes and 5 sec.

When the needle to obtain cerebrospinal fluid pressures was inserted in the lateral ventricle, an immediate pressure was obtained showing the characteristic pulse wave The tracings also fluctuated with respiratory movements when these were present. During anaphylaxis, the perilymphatic pressure rise was not simultaneous, but followed immediately after the cerebrospinal pressure rise. The perilymphatic peak was not reached until

1 minute and 10 seconds (average time) after the cerebrospinal fluid pressure peak

The cerebrospinal fluid pressure rise was sustained at the time of the perilymphatic pressure peak Both pressures then continued to decrease, keeping their pressure differences interrupted only for the second cerebrospinal fluid peak and then the pressures equalized when reaching the base line

When an injection of 0.5 ml of Ringer s solution or egg white in a non sensitized guinea pig was given intravenously this amount caused no changes in the cerebrospinal or perilymphatic pressures. Changes appear if the amount is greater than 0.5 ml For this reason the shocking dose in our animals was kept at less than 0.5 ml

D *Simultaneous perilymphatic and cerebrospinal fluid pressures in the cat* (Figs. 25 26 27)

Pressures under anaphylaxis were not studied

The average cerebrospinal fluid pressure in the cat was found to be 5.39 mm Hg.

The average perilymphatic fluid pressure was found to be 4 42 mm Hg

Several experiments were performed to study the relationship between the cerebrospinal fluid pressures and the perilymphatic pressure

1 In some experiments, over 1 ml of Ringer s solution was injected into the dural sac. This resulted in immediate rise of both the cerebrospinal fluid pressure and the perilymphatic fluid pressure almost simultaneously The relationship of the cerebrospinal to the perilymphatic pressure remained approximately the same i.e cerebrospinal fluid pressure 1 mm Hg more than the perilymph pressure Upon equilibration of the pressures, they again maintain this relationship The time for the pressures to equilibrate after injection into the dural sac appeared to be dependent upon the volume injected This in turn, may be dependent upon the reabsorption of the excess fluid The smallest volume of Ringer s solution injected in the dural sac that would produce a change in cerebrospinal fluid pressures, was found to be 0.2 ml.

2 When cerebrospinal or perilymphatic fluids were withdrawn from the cat the pressures diminished, but they were promptly compensated. It was then apparent that there was a normal physiological process which equilibrates sudden increases or losses of cerebrospinal or perilymphatic fluids in the cat

3 When the posterior extremities and the pelvis of the cat were elevated to 30 there was a rapid increase of the cerebrospinal fluid pressure but the perilymphatic pressure remained the same When the elevation was increased to 90 the perilymphatic pressure rose steadily The lack of change when the elevation was 30 suggests that a critical pressure level must be reached before affecting the perilymph pressure When the post

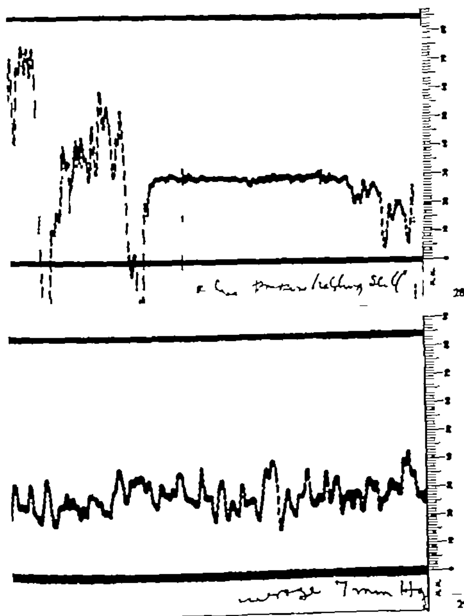


Fig 28. Perilymphatic pressure i the h man.

Fig 29 Perilymphatic pressure i the human.

tion of the cat was replaced to its original level the perilymph returned to its original pressure level

4 When the cochlear aqueduct was patent injection in the subdural space of physiological solution produced an immediate increase in the

perilymphatic fluid pressure No change occurred when the aqueduct was obstructed Methylene Blue was also utilized to confirm the occlusion of the cochlear aqueduct. When this dye was injected into the dural sac under pressure no dye was noted in the perilymphatic fluid nor did the perilymphatic fluid pressure increase under increased pressure of the cerebrospinal fluid In other words, complete obliteration of the cochlear aqueduct prevents the elevation of perilymphatic pressures secondary to cerebrospinal fluid pressure rise

5 The cerebrospinal fluid pressure in the cat was found to be 1 mm Hg higher than the cerebrospinal fluid pressure of the guinea pig The perilymph pressure in the cat was also found to be 1 mm Hg higher than the perilymphatic pressure of the guinea pig

E. *Perilymphatic and cerebrospinal fluid pressures in the rhesus monkey*

Sufficient data is not available to quote average pressures. In the two experiments performed the cerebrospinal fluid pressure was found to be approximately three times higher than that of the perilymph The experiments were not conclusive

F *Perilymphatic fluid pressure in the human*
(Figs 28 29)

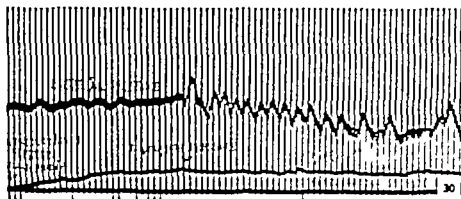
Perilymphatic pressure measurements were attempted in two patients during the course of a routine labyrinthectomy to relieve incapacitating vertigo (the ear was deaf) The apparatus was adapted for a sterile procedure The micropipette was inserted through the stapedial footplate. Pressures were obtained against a baseline difference (Fig 30)

G *Simultaneous endolymphatic and arterial pressures in the guinea pig before and during anaphylaxis*
(Figs. 30 31 32, 33 34)

The findings on these experiments were similar to those obtained in previous experiments where each of these pressures were taken simultaneously with venous or cerebrospinal fluid measurements.

H *Simultaneous endolymphatic and cerebrospinal fluid pressures in the guinea pig before and during anaphylaxis*

The values of endolymphatic and cerebrospinal fluid pressures in this series of experiments were similar to those mentioned in previous experiments. These experiments were similar to those mentioned in previous paragraphs. These experiments were done in an effort to correlate endolymphatic and cerebrospinal fluid pressure changes mainly to investigate to what extent endolymphatic pressure is dependent to cerebrospinal fluid pressure changes.



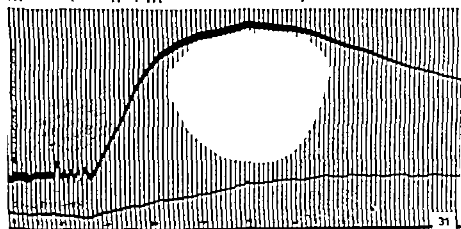




Fig. 30 Simultaneou nd lymphatic d art rial pressures during anaphylaxis; pre-shock pressure

Fig. 31 Same experiment. Intravenou introducti f shockl g dose Not arterial nd endolymph rise.

Fig. 32. Same experiment Postshock. Death of imal End lymphatic pressure reaches baselin last.

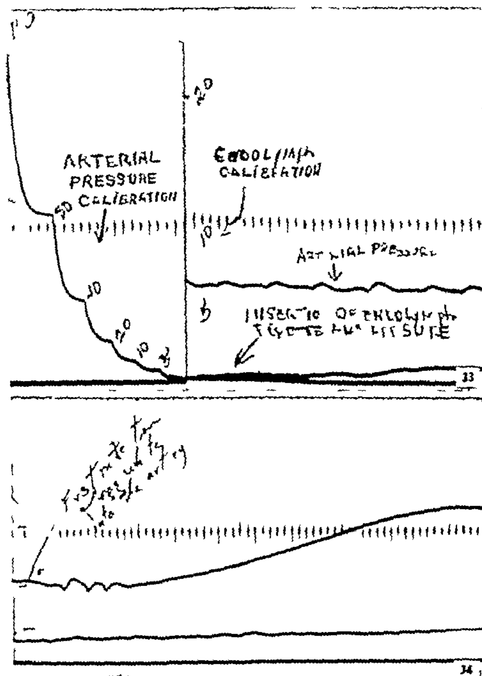


Fig. 33 Calibration hart Simultaneou endolymphatic nd rterial pressures during phylaxis.

F g. 34 Same perime t First injection f egg whit i t artery

During anaphylaxis, the endolymphatic pressure did not follow the second cerebrospinal fluid peak which had been attributed to cerebral edema No concomitant rise in endolymphatic pressure occurred during the second cerebrospinal fluid pres ure peak

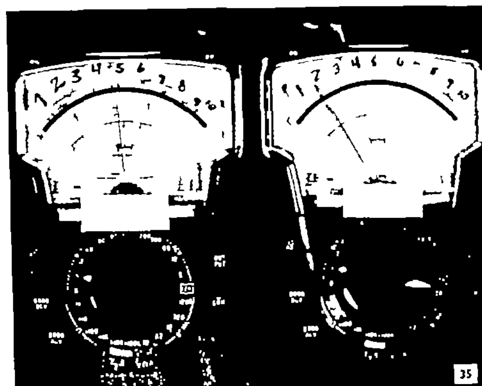
In some experiments the cerebrospinal fluid pressure was relieved by causing the cerebrospinal fluid pressure to escape to Ringer's solution. The Ringer's solution was open to atmosphere During anaphylaxis, no changes were noted in the cerebrospinal fluid pressure when the cerebrospinal fluid pressure was relieved The endolymphatic pressure increased to a lesser degree than when seen without the relief of the cerebrospinal fluid pressure

I *Simultaneous endolymphatic and perilymphatic fluid pressures in the guinea pig before and during anaphylaxis*
(Figs. 35 36)

Average endolymphatic initial (pre-shock) pressure 2 0 mm Hg
Average perilymphatic initial (pre shock) pressure 3.5 mm Hg
Difference between endolymphatic and perilymphatic pressures 1.5 mm Hg
Variations of endolymphatic pressure from 1.3 mm Hg to 3.2 mm Hg
Variation of perilymphatic pressure from 2.2 mm Hg to 6 6 mm Hg
Average endolymphatic peak pressure during anaphylaxis 3 4 mm Hg.
Average perilymphatic peak pressure during anaphylaxis 6.2 mm Hg
(Difference in pressure peaks between endolymph and perilymph 2.8 mm Hg)
Variations in peak pressures during anaphylaxis
Endolymphatic from 2.2 to 5.8 mm Hg
Perilymphatic pressure from 4 0 mm Hg to 10 mm Hg
Average peak time Endolymph 6 minutes—Perilymph 3 minutes.
Variations in endolymphatic peak time from 4 min and 30 sec to 8 minutes.
Variations in perilymphatic peak time from 2 minutes to 4 minutes.

The simultaneous pressure measurements of endolymph and perilymph resulted in similar pressure findings as when endolymph or perilymph were taken individually Two different methods of recording these pressures were utilized It was possible to identify the pressures obtained by examination of their tracings The manner in which these pressures appeared when the micropipette was introduced was characteristic In the case of the perilymph, pressures registered immediately and gave higher readings. Perilymphatic pressures were also subject to more variability since they were more easily affected by blood pressure and cerebrospinal fluid pressure changes. The endolymphatic pressure appeared gradually after the insertion of the pipette, and then stabilized and remained more stable than the perilymph Endolymphatic readings were also lower than those of the perilymph

During each experiment certain tests were made in order to determine whether one was measuring true endolymph and perilymph and to ascertain that there were no communications between the endolymphatic and the perilymphatic fluid (1) application of suction to the bulla without touching it produced no change in the voltmeter thus indicating no leak,



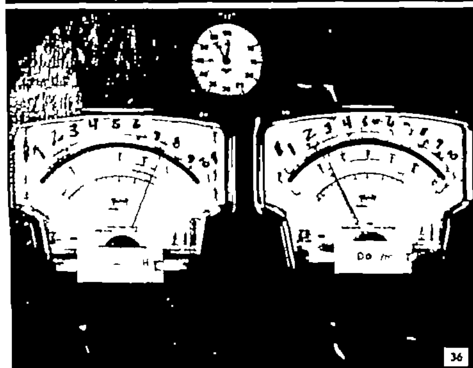


Fig 35. Preshock imultaneous end lymphati and perilymphatic pressures. G inea pig Large umbers indicat millimeters of mercury N te diff rence in pressure

Fig 36 End lymphatic nd perilymphatic pressures during anaphylaxis. Sam experiment in Fig. 35 Note increase bo h pressures.

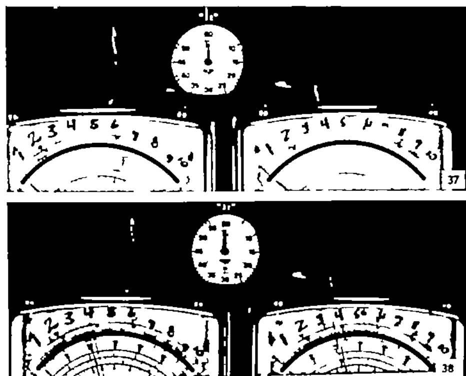


Fig 37 N t needle t O Scal media and scala tympa l. When on pipett 1 pe 1 tmosphere the pressures registered on both meters are lost. Thi indicates comm ica- tio between perilymphatic nd d lymph tl paces.

Fig. 38. Not both needles indicati g same pressure The nd lymph pipette ha perforated R issner' membrane llowi g the pressures t equilibrat

(2) the speed with which the increase in pressure was recorded on the meters was greater with the perilymphatic than with the endolymphatic fluid (3) when one pipette was opened to atmosphere the output meter belonging to the other pipette showed no change. If there was a change and the pressure of the other voltmeter fell, it then indicated that the Reissner s or basal membranes were ruptured (Fig. 37) Communication between the endolymph and perilymph spaces would equalize the pressures (Fig 38) (4) the perilymphatic pressure showed more fluctuations and greater sensitivity changes especially following changes in venous, arterial and cerebrospinal fluid pressures. During anaphylaxis, the increased pressure of the perilymph was more sudden, reaching its peak 3 minutes after the shocking dose During anaphylaxis, the endolymphatic pressure rise had its onset at the same time as the other fluids. However the pressure rise was more gradual Endolymph peak pressure is reached six minutes after the shocking dose This was later than any of the other fluid pressures measured

Once the perilymphatic pressure reached its peak, it began to decrease rapidly due to shock. At a point about 8 minutes from the shocking dose

both the endolymphatic and perilymphatic pressures equilibrate The perilymphatic measurements then fell below the values of endolymph When the animal recovered from the anaphylactic shock, the endolymph still remained higher than the perilymph These observations were carried for over an hour from the shocking dose When the animal dies, the perilymphatic pressure reaches zero before the endolymph reaches this level The endolymph maintained its pressure from 10 to 60 seconds after the perilymph had reached the zero line When the animal survives, and a second shocking dose is given, a slight increase develops in both the endolymphatic and perilymphatic pressures however there was no gradual drop as seen with the first shocking dose It can be assumed that in this case the animal usually did not go into a second shock. During the recovery period of the animal the perilymphatic pressure continued to increase over the endolymphatic until their initial relationship was recovered

Simultaneous endolymphatic and perilymphatic fluid pressures before and during anaphylaxis

Sample experiment I

Time (min)		Perilymph (mm. Hg.)	Endolymph (mm. Hg.)
0	Initial pressures	4 20	1 67
	Egg white 0.3 ml. i.v		
3.4		(*)*7 47*	
5			4 0
6		6.67	4.17
		6.93	4 40
8		6.67	4 47
9		5.74	*4.87*(*)
10		4.80	4.57
11		*4 87*	*4.87*( )
12		(*)3.43	4.57
13		2.26	4.54
14		2.0	4.33

Time (min)	Perilymph (mm. Hg.)	Endolymph (mm. Hg.)
15	1 67	4 13
16	1.87	4 00
17	1.87	4.00
18	1 74	3.80
19	1 93	3.73
20	2.00	3.60
21	2.07	3.60
22	2.20	3.53
23	2.26	3.47
24	2.33	3.53
25	2.26	3.47
26	2.40	3.40
27	2.47	3.33
28	2.40	3.33
29	2.40	3.27
30	2.40	3.33

Animal survived
Experiment terminated
Pipette clear

Highest perilymph pressure.
* Highest endolymph pressure
Equal pressure

From this point perilymph decreased more rapidly due to shock; endolymph remains higher than perilymph during shock.

Sample experiment II

Time (min)		Perilymph (mm. Hg.)	Endolymph (mm. Hg.)	Time (min)		Perilymph (mm. Hg.)	Endolymph (mm. Hg.)
0	Initial pressures	3.17	1.33	20		3.60	3.33
	Egg whit 0.2 ml. Lv			21		3.53	3.33
1		5.31	2.0	22	(*)	3.46	3.46
2		(*)4.66	2.67	23		3.20	3.20
3		6.00	3.07	24		2.53	2.60
4		5.31	3.20	25		1.74	2.26
5		5.00	3.26	26		1.20	2.20
6		4.80	3.33	27		0.87	1.60
7		4.67	3.26	28		0.67	1.40
8		4.67	3.33	29		0.60	1.33
9		4.40	3.40	30		0.40	1.07
10		4.40	3.26	31		0.33	1.00
11		4.26	3.33	32		0.26	0.93
12		4.33	3.46	33		0.20	0.80
13		4.27	3.40	34		0.13	0.67
14		4.27	3.46	35		0.13	0.67
15		4.00	3.53(*)	36		0.00	0.54
16		4.00	3.53	37		0.07	0.60
17		3.39	3.46	38			0.54
18		3.87	3.46	39			0.54
19		3.71	3.46	40			0.40
				41			0.26
				42			0.20
						Animal died	0.00

Highest perilymph pressure.
Highest endolymph pressure.
Equal pressures.



Fig. 39 G lne pig cochlea Control experim nt N dil tatio f scala media In vi fixati

this may suggest the possible existence of a protective mechanism at the cochlear aqueduct to maintain this pressure difference. The endolymphatic pressure may also have a protective mechanism within the scala media, in the form of a pressure receptor or contractile element Such an element could be similar to the capillaries in the kidney which are believed to have a contractile function in helping to regulate the pressure within the glom eruli

The studies in simultaneous cerebrospinal fluid and endolymphatic fluid pressure during anaphylaxis revealed that the endolymph continued to increase its pressure when the cerebrospinal fluid pressure was relieved This would indicate that there was a mechanism apart from the cerebrospinal fluid changes that would produce this increase This mechanism may possibly be related to venous pressure Endolymph may then appear to be more directly related to venous pressure than to cerebrospinal fluid pressure

Hallpike and Ledoux (24) have reported the osmotic pressures of the



Fig. 40 Guinea pig cochleaduri g anaph lactic shock. In vi fixati n. Micropipettes not inserted Not dil tati n f scala media (These sections prepared nder grant from th Deafness Research F undati)

endolymph and perilymph to be equal but greater than those of the cerebrospinal fluid and the blood. The findings of Weille *et al* (40) with in dividually obtained endolymph and perilymph pressures, together with the results of the same pressures simultaneously obtained and reported in this presentation, indicate a higher perilymphatic than endolymphatic pressure Considering these findings, the endolymph would appear to originate from two mechanisms

by filtration as well as by secretion

The filtration could occur from the scala vestibuli through Reissner's mem brane Th secretion as well as electrolite exchange from the stria vascularis. According to Guild (19) the circulation will follow a direction to the saccus endolymphaticus, where excess fluid will be reabsorbed. This structure may serve also to regulate sudden changes in endolymphatic pressure provid ing that no obstruction exists between it and the cochlea.

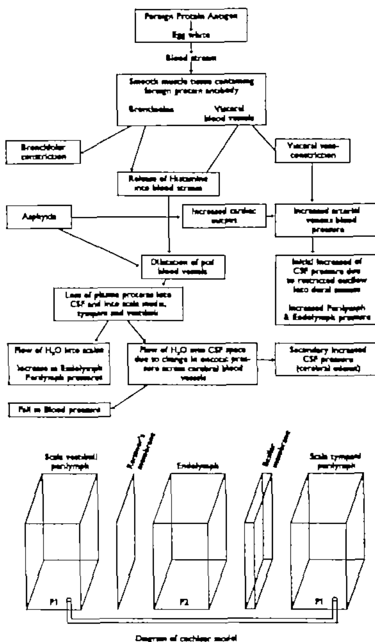


Diagram of cochlear model

B *Anaphylactic shock*

In order to explain the phenomena observed, the following chain of events is proposed

The initial sensitizing dose of albumin acts as an antigen agent against which the guinea pig forms antibodies

It is suggested in the literature that these antibodies reside in the smooth muscle of the visceral vasculature. When, after 21 days of the initial sensitiz ing dose, the antibody level was at its highest point, a further dose of antigen (egg white) was injected and anaphylactic shock took place

Anaphylaxis produces a generalized release of histamin and bradikinin therefore a localized liberation of these substances in the stria vascularis. An initial contraction of the arterioles and pre-capillary sphincters occurs, followed by a dilatation of the venules and arterioles as reported by Weille, Martinez and Irwin (38) This results in local venous stasis and diminished capillary flow

The intercellular junctions of the capillary endothelium widen and the basement membrane ruptures, resulting in increased capillary permeability Plasma proteins and electrolites then migrate into the scala media, tympani and vestibuli. The osmotic pressure of these fluids is probably increased Consequently fluid is taken and this results in an increase of perilymphatic and endolymphatic pressures The concentration of proteins and electrolites is probably greater in the scala media due to the numerous blood vessels present in the stria vascularis. The release of protein from the capillaries may cause an increase of fluid transfer into the same capillaries, resulting in an added increase of venous pressure In the cerebrospinal fluid, this results in sufficient transfer of water across the vessel walls to give a secondary increase in cerebrospinal fluid pressure (cerebral edema) as well as a drop in blood pressure

The systemic effect of anaphylaxis results also in the contraction of the smooth musculature of the bronchioles. This causes asphyxia which con tributes to the increased cardiac output The increased cardiac output and a generalized initial visceral vasoconstriction caused by the release of histamine, produces an increased arterial and venous blood pressure The increased blood pressure then restricts the flow of the cerebrospinal fluid into the dural sinuses which causes a rise in the cerebrospinal fluid pres sure and a concomitant increase of perilymphatic and endolymphatic pressures The general release of histamine into the blood stream, together with asphyxia, causes further dilation of the pial, spiral ligement and stria vascularis blood vessels. PH, pO2 and pCO2 determinations of carotid artery blood samples were done at one minute intervals from the injection of the shocking dose. No changes occured until after 6 minutes from shock. Con sequently the initial increase of endolymphatic and perilymphatic pressures is believed to be produced by the anaphylactic reaction and not initially by asphyxia.

The increased endolymphatic and perilymphatic fluid pressures in these experiments, would seem to be due to several factors

1 An increase in venous, arterial and cerebrospinal fluid pressures.
2 Increased osmotic pressure.
3 Electrolite exchange
4 Possible increase of endolymphatic and perilymphatic fluid production.

It wo ld seem possible that repeated, localized, allergic or inflamatory change w hin the cochlear duct, may result in a permanently dilated scala media, uch as in human endolymphatic hydrops.

The fact that the stria vascularis is present only in the cochlear duct, may explain the absence of recognized pathology in the semicircular canals of human temporal bones with the clinical diagnosis of Menière's disease Further work is needed to determine, possibly by electron microscopy whether any pathology is present in the strial vessels, basal membrane or endothelium A study of possible obstruction to the circulation or absorption of the endolymph is equally necessary

IV SUMMARY

A method to obtain simultaneous endolymphatic and perilymphatic fluid pressures in the guinea pig under anesthesia has been described This technic has been adapted to obtain simultaneous cerebrospinal fluid arterial, and venous pressures in the guinea pig, in the cat and in the rhesus monkey

A system has been devised to corroborate the characteristics of the hydraulic system obtaining these pressures.

The entire hydraulic and electronic systems have been calibrated by means of a water manometer

Anaphylactic shock has been produced in anesthetized guinea pigs previously sensitized by egg white Observations have been made of the changes in pressure occurring before and during anaphylaxis in the endolymphatic and perilymphatic fluids. Their association with changes in cerebrospinal fluid arterial and venous pressures has been studied.

A discussion of a possible physiopathological method producing these changes has been presented

The pressures obtained were those found with two different electronic systems. The results with each of these systems were similar They may not necessarily represent the actual pressures within the cochlea. However the relationship found among the different fluids before and during anaphylaxis may be a significant finding

Endolymphatic and perilymphatic fluid pressures behave as two different systems. Perilymph reacts more quickly to changes in cerebrospinal fluid, venous and arterial pressures The endolymph is not as easily affected by mechanical factors. It reacts to physiological changes as in the case of anaphylaxis

When the basilar or Reissner's membranes were ruptured by the micropipettes, endolymphatic and perilymphatic pressures became equalized Upon the death of the animal or the removal of the pipettes, the pressures returned to baseline.

The cerebrospinal fluid pressure in the cisterna magna and in the lateral ventricle of the guinea pig was found to be equal

Perilymphatic fluid pressures in the guinea pig and in the cat were found to be approximately one millimeter of mercury lower than cerebrospinal fluid pressures. The latter is of interest in view of the fact that the cochlear aqueduct in the cat is thought to be widely patent

Investigations of cerebrospinal and perilymphatic pressures in the guinea pig, cat and rhesus monkey indicate the variability of patency of the

cochlear aqueduct according to the different animal species. The transmission of pressure changes from the cerebrospinal fluid to the perilymphatic fluid was more readily obtained in the cat. This may indicate a wider cochlear aqueduct. This transmission is less evident but still present in the guinea pig and the rhesus monkey.

The cerebrospinal fluid pressures obtained from the lateral ventricle of the brain in the guinea pig were found to be positive when placing the guinea pig in the erect position. Injection of more than 0.5 ml of isotonic solution intravenously in the guinea pig produced transient rise in cerebrospinal and perilymphatic fluid pressures. The endolymphatic pressure was not affected. The rise of the former pressure is probably related to an increased blood volume. Injections in the dural sac produced more marked changes in the cerebrospinal and perilymphatic pressures.

Endolymphatic and perilymphatic fluid pressures increased during anaphylaxis. The endolymph increased almost one time its original pressure. The perilymph increased three times its original pressure. This pressure increase developed more rapidly in the perilymphatic pressure than in the endolymphatic pressure.

The cerebrospinal fluid increased twice its original pressure during anaphylaxis. Two peaks were noted. The first peak appeared to be secondary to arterial and venous pressure changes. The second peak appeared to be secondary to cerebral edema.

Venous and arterial pressures also increased during anaphylaxis. Arterial pressure increased more sharply.

After obtaining a pressure peak during anaphylaxis, each of the fluid pressures studied began to decrease. Perilymphatic pressure decreased much faster and eventually registered less pressure than the endolymphatic fluid. If the animal recovered from the anaphylactic shock, the pressures were re-established. When the animal died, all pressures disappeared. Endolymphatic pressure was the last to reach the baseline. This was probably due to its increased osmotic pressure after anaphylaxis.

The equalization of pressure between the endolymphatic fluid and the perilymphatic fluid during anaphylaxis may be due to increased permeability across Reissner's membrane as well as to decreased perilymphatic pressure due to shock.

Endolymphatic fluid changes during anaphylaxis are to a great extent directly related to increased venous, arterial and cerebrospinal fluid pressure changes. When the cerebrospinal fluid pressure is relieved, it does not prevent the increase of endolymphatic pressure during anaphylaxis. It would seem that the endolymph was more dependent on venous pressure.

The perilymphatic pressures showed dependency to both cerebrospinal and venous pressure changes.

Endolymphatic and perilymphatic fluid pressures appear to be, to a certain extent related to venous, arterial and cerebrospinal fluid pressures

in this order of importance The endolymphatic pressure appears also dependent on its own pressure formation Other factors such as osmotic pressure electrolyte exchange and CO_2 tension in the blood probably play an important role in the variations of these pressures.

Possible changes in perilymphatic and endolymphatic fluid pressures due to diastolic and systolic blood pressure changes could not be established because of the long time constant (12.0 sec) of the hydraulic system The pressures obtained are mean pressures

The utilization of the pressure simulator to calibrate the hydraulic system demonstrated that the hydraulic dampening effect through the pipette and plastic tubing connectors was negligible

From the experiments described it may be possible to suspect a circulation of fluid from the scala vestibuli into the scala media and thence into the saccus endolymphaticus.

SUMMARY OF PRESSURE RESULTS

Preshock pressures (mm. Hg.)		Pressures during anaphylaxis (max. peak) (mm Hg.)	Variations	
			Preshock (mm. Hg.)	Anaphylaxis (mm. Hg.)
Endolymphatic	2.0	3.4	1.3–3.2	2.2– 5.8
Perilymphatic	3.5	6.2	2.2–6.6	4 0–10 0
Cerebrospinal fluid	4.5	9.0 1st peak 8.6 2nd peak		
Venous	3.5	9.0		
Arterial	28.8	4 .5		

Time of occurrence of the different pressure peaks during anaphylaxis from injection of the shocking dose

	min	sec
Arterial pressure	1	24
Venous pressure	1	25
Cerebrospinal fluid	1	50 (1st peak)
	8	5 (2nd peak)
Endolymph	6	20
Perilymph	3	

The duration of the endolymphatic pressure rise varied from 1 mim 55 seconds to 10 minutes at the removal of the pipette The first p to reach its maximum peak during anaphylaxis was the arterial The last pressure to reach its maximum peak during anaphylaxis w endolymph.

The cerebrospinal fluid pressure was found to be an average of higher than the perilymph and 2 6 mm higher than the endolymph

The perilymph was found to be 1.5 mm Hg average higher than tl lymph

The perilymph pressure and venous pressures were found to be

V CONCLUSIONS

Endolymphatic fluid pressures in the guinea pig were found to be an average of 1.5 mm Hg less than perilymphatic pressures before anaphylaxis

Perilymphatic and venous pressures were found to have the same pressure measurements. Cerebrospinal fluid pressure measurements were found to be on the average of 1 mm Hg greater than perilymphatic pressures. A direct communication between these fluids would then appear unlikely

Endolymphatic and perilymphatic fluid pressures were unobtainable when the animal died

Variations in cerebrospinal fluid pressures were transmitted to the perilymphatic fluid once the change reached a certain pressure level The pressure difference between these fluids remained unchanged Pressure measurements appear to indicate a more readily cerebrospinal fluid pressure transmission through the cochlear aqueduct in the cat than in the guinea pig or the rhesus monkey

Changes in venous and arterial pressures were transmitted to the cerebrospinal and perilymphatic fluids. The endolymphatic pressure appears more resistant to change when above pressures were increased mechanically Endolymphatic and perilymphatic fluid pressures became equalized when Reissner's or the basilar membranes were ruptured by the introduction of the micropipette.

All pressures studied increased during anaphylactic shock. The scala media was found dilated in all histological sections when the micropipettes were not inserted.

Endolymphatic fluid pressure changes during anaphylaxis appeared to be the result of the following factors in this order of importance

1 Increased venous pressure
2 Increased endolymphatic fluid formation
3 Increased osmotic pressure
4 Increased cerebrospinal and arterial pressures

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SUPPLEMENTUM 239

EXPLORATION OF VESTIBULAR DAMAGE IN GUINEA PIGS FOLLOWING MECHANICAL STIMULATION

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UPPSALA 1968

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Dedicated to the Memory of
Stacy R. Guild

CONTENTS

ABSTRACT

Guinea pigs were examined with behavioral tests, gross dissection, and celloidin serial sections of the temporal bone following exposure to either linear acceleration, vibration, or loud sound. The major results of these investigations are as follows (1) Minimum acceleration intensity for loss of the righting reflex and swimming ability is approximately 50 G applied for 60 sec. Loss of otoconia from the maculae may be produced by acceleration as low as 12–25 G for 195–330 sec. Acceleration at 100 G for 30 sec results in severe loss of otoconia from all maculae (2) Although the stimulus intensity required to produce evidence of behavioral loss is greater than the stimulus intensity which results in structural damage, ability to perform the righting reflex and otoconia loss ratings are highly correlated ($r = -0.69$) (3) Recovery of swimming ability and the righting reflex may take place from 1 to 64 days following exposure to accelerations of up to 300 G for 15 sec. Exposure to 400 G for 15–20 sec results in irreversible loss of the righting reflex and severe disturbance of swimming ability No histological evidence for otoconia reformation during the postexposure period was obtained, but the possibility of replenishment of the gelatinous layer is suggested (4) Orientation of the acceleration vector perpendicular to the macular surface produces greater evidence of damage than orientation of the acceleration vector parallel to the macular surface. (5) Vibration at 1–2 G (peak) 6–8 Hz for 6 hours may produce transient behavioral loss and slight displacement of otoconia. (6) Exposure to noise at 154 dB SPL for 20 min results in no evidence of behavioral loss and minor otoconia dislocation.

INTRODUCTION

This paper is concerned with the effects of intense mechanical stimulation on the vestibular system. Exploration of stimulus parameters that produce permanent damage to the vestibular apparatus has been our major interest. In the course of these studies guinea pigs have been exposed to (1) linear acceleration (2) vibration, and (3) loud sound. Assessment of vestibular damage following exposure to these stimuli has been accomplished with (1) behavioral techniques, (2) gross dissection, and (3) histological examination of celloidin serial sections of temporal bones.

Studies of vestibular damage are of theoretical as well as practical interest. From a theoretical view determination of thresholds for structural damage with a variety of stimuli should lead to a more complete understanding of the dynamics of vestibular stimulation, particularly with regard to functional characteristics of the various components and sections of the vestibular apparatus. From a practical view exposure of human beings to unusual acceleration environments during aerospace missions and as a result of accidents indicates the necessity for obtaining information about the response of the vestibular apparatus to intense stimulation. The experiments discussed in this paper have relevance for the following aerospace operations vibration as a result of buffeting during low-level operations in high performance aircraft, vibration and acceleration during lift-off of aerospace vehicles, and repeated exposure to high acceleration during astronaut training.

Literature

Although the effects of intense mechanical stimulation on the vestibular apparatus have been the subject of several investigations during the past six decades, man's recent entry into the new environments of mechanical stimulation noted above indicates the need for extending previous observations.

Linear acceleration

Studies of vestibular damage following intense linear acceleration were first undertaken by Wittmaack (1909). He demonstrated complete removal of the otoconia and gelatinous layer following rotation of guinea pigs around the cephalo-caudal axis at speeds of 2000 rpm for periods of 45–60 sec. The linear acceleration produced by this stimulus was probably in the region of 50–100 G. Wittmaack was also the first to suggest the possibility of

reformation of the otolithic membrane following its removal by centrifugation.

Wittmaack's observations were confirmed and extended by a number of investigators including Hasegawa (1931) and de Kleyn and Versteegh (1933). The studies by Hasegawa are of particular relevance to the present investigation. With his investigations Hasegawa attempted to define the relationships between behavioral and anatomical loss. He found that the threshold for behavioral loss was approximately 290 G although anatomical damage could be detected at somewhat lower stimulus intensities.

Recently investigations of the effects of intense linear acceleration have been performed by Margaria, Gualtierotti, and Spinelli (1958) and Spoendlin, Schuknecht and Graybiel (1965). Margaria *et al* report that the threshold for detachment of the otoconia in fish and frogs is approximately 150 G applied for 1 min. Spoendlin *et al* were unable to find any evidence of structural damage in the vestibular area with either light or electron microscopy following exposure of squirrel monkeys to 10.9 G for periods up to 10 min.

The present authors previously demonstrated clearly (Parker, von Gierke, and Covell 1965) that vestibular damage takes place when guinea pigs are subjected to linear acceleration of 200–400 G for periods of 8–12 sec in a previous paper. Both histological and behavioral techniques were employed in those damage determinations. Further experimentation revealed no evidence of behavioral damage and slight to moderate otoconia displacement following impact decelerations of 240–314 G.

Vibration

Riopelle, Hines, and Lawrence (1958) reported that monkeys which were exposed to 2.6 G (peak) sinusoidal vibration at 10 Hz for 8 hours demonstrated no behavioral loss in terms of jumping ability and pattern discrimination tests. Histological observation of a group of monkeys which received similar vibration exposures revealed slight evidence of structural damage in the form of partial detachment of the otolithic membrane for three of six animals observed.

Loud Sound and Blast

The development of modern technology has given rise to increasingly powerful noise sources. Equilibrium as well as auditory difficulties have been reported following exposure to loud sound. The considerable literature relating sound stimulation to vestibular excitation has been reviewed by Dickson and Chadwick (1951).

Investigations into the possibility of producing vestibular damage by exposure to loud sound have been reported by Rüedi and Furrer (1946), Ades (1953), McCabe and Lawrence (1958) and Albernaz, Covell and Eldredge (1959). Rüedi and Furrer report a case of severe equilibrium disturbances following the accidental exposure of an officer to a hand grenade explosion

at a distance of 2.2 m Further observations with guinea pigs demonstrated the presence of damage in the vestibule and semicircular canals following exposure to an explosion of 5 kg Trotyl at a distance of 5.5 m Ades noted transient deficiencies of the righting reflex and posture in a cat following exposure to a pure tone of 960 Hz at 140 dB SPL. The papers by McCabe and Lawrence and Albernaz *et al* report rupture of the membraneous partition and the saccule following exposure to noise or pure tones as intensities of 140–153 dB SPL.

Summary

Each of the three areas reviewed reveals unanswered questions which, in view of the increasing importance of these problems, were deemed worthy of further investigation. Studies of the effects of linear acceleration had not clearly defined the thresholds for behavioral loss or physical destruction. However the behavioral techniques previously employed were not easily quantified, and judgement of damage with these techniques is, in our experience somewhat difficult. The area of vibration research was clearly in need of continued investigation in terms of extending the range of stimuli and the behavioral techniques used to evaluate the exposures. The effects of loud sound had received careful attention on the anatomical side, but behavioral observations were sparse In view of the above gaps in our knowledge the present series of investigations was undertaken

Experiments

A series of somewhat diverse experiments, undertaken to explore vestibular response to intense mechanical stimulation will be described. These experiments include

- I Effects of linear acceleration
  - a The relationship between exposure duration and peak linear acceleration for the production of vestibular damage
  - b. Long-term recovery
  - c Orientation of the maculae with respect to the acceleration vector
  - d. Linear acceleration combined with axial rotation
- II Effects of vibration.
- III Effects of loud sound

Experiment I a. Linear Acceleration Relationship Between Exposure Duration and Peak Acceleration

Purpose

This experiment was designed to investigate the relationship between exposure duration and peak acceleration for trapezoidal G-time exposure histories in the production of vestibular damage If the otoconia and sup-

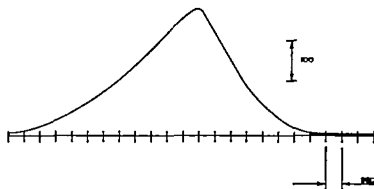


Fig. 1 Acceleration profile for Space Flight Acceleration Profile Simulat bearing guine pig. The profil has lines acceleration peak f 410 G, rise tim f 11.5 sec and decay time of 8.3 sec. The maximum angular acceleration associated with this run was about 580°/sec²

porting cells are assumed to be a simple mass-spring system two types of damage threshold effects would be expected (1) minimum peak ac celeration for long exposure times, and (2) minimum velocity change or area under G-profile for short exposure durations. The purpose of this investigation was to determine a duration-peak acceleration damage threshold curve.

Method

Subjects

Only guinea pigs which were judged normal were used in this investigation. For our purposes, normality was defined in terms of the following generally alert and active appearance, freedom from nasal or ocular discharge, strong pinna reflex, clear tympanic membranes, and ability to per form the righting reflex at least eight times in ten drops. Data from 42 guinea pigs which were run on five separate occasions were compiled to provide a picture of the relationship between exposure duration and peak G in the production of vestibular damage

Apparatus

The apparatus employed to accelerate the subjects, the Space Flight Ac celeration Profile Simulator (SFAPS) has been described in previous articles (Pine and Barr 1963 Parker von Gierke and Covell, 1965)

Essentially the SFAPS is a centrifuge which consists of a 92-cm primary arm, rotational center mass balance payload capsule, motive power source support structure and instrumentation to detect the acceleration profile Special design features allow onset and decay rates up to 40 G/sec. An ex ample of the acceleration profiles obtainable with this device is presented in Figure 1

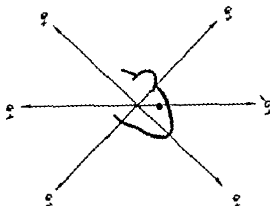


Fig 2 Orient tion of the subject with respect t th f rces generated by the vibration exciter or Space Flight Accelerati n Profil Simul tor The arrows represent the inertial resultant f the head acceleration, and otoconia displacement f the ri us directions would be as f ll w +G_x nose t neck; −G_x neck t nose; +G_z jaw t ey −G_z ey to j w; +G_y left t right; −G_y right t left.

Exposure and behavioral assessment procedures

Before and after exposure the experimental animals were subjected to tests of the righting reflex and in most cases, swimming ability in order to obtain an estimate of vestibular capability With this procedure each animal served as his own control

The phrase "righting reflex" refers to the complex series of movements whereby a blindfolded animal which is dropped in an inverted position achieves a four point landing Before exposure the animals were able to right themselves at least eight times in ten drops when dropped from heights of 25–30 cm. Swimming ability was determined by placing the guinea pigs in a 45 by 100 cm tank which was filled with water to a depth of 25 cm. Normal guinea pigs are excellent swimmers they will move in straight lines unless presented with a barrier and will surface readily when submerged

For each run on the SFAPS the subject was placed in a coffin-like box which provided dorsal support The subject s nose was taped to a molded headpiece which assured proper alignment of the temporal bones during acceleration All animals were subjected to acceleration in the +G orienta tion as illustrated in Figure 2

Acceleration profiles employed in this series of experiments are sum marized in Table 1 Twenty two animals were exposed to peak accelerations of 100–400 G over triangular acceleration profiles of 9–21 sec. Profiles which gave a minimum velocity change, or area under the acceleration profile at each peak acceleration level were selected subject to the apparatus onset and decay rate limitations noted in Figure 3

In two series of runs which were designed specifically to attack the problem of long exposure duration 20 animals were exposed to a somewhat

modified acceleration profile. After the initial rise acceleration was main tained at a specified peak G for varying periods. These periods were deter mined after consideration of the area under the acceleration profile which may be presented in G-sec. An attempt was made to determine threshold for behavioral loss in terms of the number of G-sec as well as peak G

Behavioral observations were performed before exposure Behavioral determinations were not made until 24 hours after exposure to eliminate transient effects.

Anatomical procedures

Gross dissection The temporal bone was examined using the techniques of gross dissection following suggestions by Engstrom and Spoendlin. Care ful removal of the stapes footplate and surrounding bone affords the ex perimenter an excellent view of the membranous labyrinth and it is a simple matter to determine major otoconia displacement under low magnification

Histology Selected guinea pigs were anesthetized with veterinary nem butal and the thorax opened. A cannula was inserted into the aorta through the left ventricle and blood was washed out with physiological saline This was followed by fixative (Heidenhain Susa) The temporal bones were then removed and put in more fixative for 16 hours. This was followed by 95% alcohol for 16–24 hours after which each bone was placed in 3% HCl and changed daily for 3–5 days or until decalcification had been completed. The specimens were then washed for 24 hours in running water dehydrated in different grades of alcohol (50 70 80 95% and absolute) for 12 hours each, then immersed in absolute alcohol and ether (equal parts) for 6 hours, after which they were put into 2, 4 8, and 12% colloidin for 3 days each, and finally imbedded in 12% celloidin. Sections were cut serially at 15 microns and every fifth section was stained with Harris hematoxylin and eosin and mounted in Canada Balsam. Occasionally it was necessary to stain and mount intervening sections when additional information was needed

Degree of otoconia loss was rated on a scale of 0 to 5+ in the following manner Complete loss of otoconia from either a macula of the saccule or the utricle was judged as grade 5+ Incomplete losses varying in amount of the otoconial layer present ranged from 4+ to 1+ Grade 4+ loss was used to indicate an almost complete loss. The remaining otoconia could have been displaced at the time of the exposure and a few finally settled on the macular surface, or as in some specimens only one small area of a macula appeared to have retained otoconia. Grade 3+ represented an in-complete loss which usually appeared to affect the whole suface of a macula or a spotty complete loss with normal distribution over intervening areas Grade 2+ represented a dispersion of the usual compact layer with some obvious loss of otoconia while grade 1+ was used to designate dis-

TABLE 1 *Behavioral loss as a function of exposure duration and peak G*

Animal	Peak G	Acceleration profile			Area under acceleration profile G-sec	Righting loss 24 Hrs
		Rise	Dwell sec	Fall		
IX-7	12	5	330	5	4020	–
IX-8	12	5	330	5	4020	–
X-5	25	5	95	5	2500	–
X-6	25	5	95	5	2500	–
IX-5	25	5	195	5	5000	–
IX-6	25	5	195	5	5000	–
XI-1	45	5	39	6	2015	–
XI-2	45	5	40	6	2047	–
V-3	50	5	45	5	2500	–
X-4	50	5	45	5	2500	–
IX-1	50	5	45	5	2500	+
IX-2	50	5	45	5	2500	–
IX-3	50	5	70	5	3750	–
IX-4	50	5	70	5	3750	+
XI-3	65	5	25	6	2075	–
XI-4	65	5	26	7	2079	–
XI-5	90	7	5.5	7	1129	–
XI-6	95	6	11	7	1690	–
XI-7	100	5	7	6	1225	+
XI-8	100	7	10	8	1750	–
I-1	100	5	0	4	450	–
X-1	100	5	20	5	2500	+
X-2	100	5	20	5	2500	+
I-2	105	5	0	4	423	–
XI-9	105	6	13	7	2075	+
XI-10	105	6	14	7	2180	–
I-3	185	8	0	6	1325	+
I-4	200	7	0	6	1200	–
II-1	200	7	0	5	1200	–
II-2	210	7	0	5	1260	–
II-6	205	7	0	5	1230	+
IX-2	200	7	0	5	1200	+
II-4	295	9	0	7	2310	+
II-8	300	9	0	6	2250	+
II-3	310	9	0	7	2400	+
IX-3	315	9	0	8	2520	+
IX-4	320	10	0	7	2720	+
I-5	397	12	0	9	3668	+
I-6	410	12	0	8	4100	+
II-7	410	11	0	8	3695	+
II-9	425	12	0	8	4250	+
IX-6	420	12	0	9	4410	+

+ Observations indicate behavioral loss (< 8 successful rightings in 10 drops)
– Observations indicate no behavioral loss.

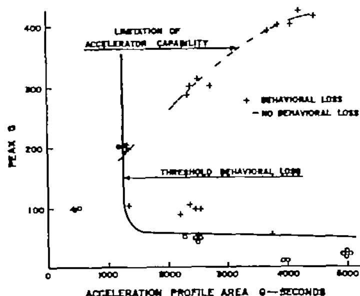


Fig 3. Behavioral los function of exposure duration and peak G The ordinat indicates peak ccleratio for the partical profile The abscissa indicates the rea under the acceleration profile in terms of G-seconds. The + signs indicate the exposure coordinates for animals which exhibited loss f the righting reflex 24 hrs fter acceleration exposure, and the – signs indicat the coordinates for the imals which exhibited no beha ioral loss. They heavy black line represents the pproximate threshold for behavioral less. The light dashed line indicates the rise and decay time limitation for the SFAPS t particular peak G lev ls.

persion and rearrangement of the individual otoconia without apparent loss.

For many maculae the grading varied for different areas as it was not unusual to have a loss from an anterior one-half with only a slight loss from the posterior one-half For this reason and also because the five grades of otoconial loss overlapped for many observations, it was decided to group the five grades into I II or III with respect to each macula. Grade 5+ then fell into group I grades 4+ and 3+ into group II, and grade 1+ and 2+ into group L If a macula of a utricle was 5+ for the posterior one-half and 2+ or 1+ for the remaining one-half it was considered as falling in group I

Results

Behavioral observations

The results of experiments on the relationship between duration of acceleration exposure and peak G are presented in Table 1 The data from Table 1 are plotted in Figure 3 for ease of conceptualization.

Animals which were able to perform successfully the righting reflex less than six times in ten drops 24 hours after exposure were classified as

demonstrating behavioral loss. Employing this cut-off the probability of classifying a normal animal as indicating behavioral loss is approximately 0 03 This is calculated on the assumption that the probability that a normal animal will fail to perform the righting reflex on a given drop is 0.2 and that there are no second order effects during the series of 10 drops. These assumptions are not strictly true and our probability of 0 03 is only a rough estimate.

Figure 3 indicates that damage is produced at the 300 and 400 G levels by the shortest acceleration profiles within the capability of the apparatus (16–20 seconds) The integrals over the acceleration profiles which have peaks between 295 and 425 G range between 2250 and 4410 G-sec.

Three of six animals exposed to accelerations in the 200-G range manifested behavioral loss Area measures associated with the 200-G peaks varied between 1200 and 1325 G-sec.

Three of the ten animals exposed to approximately 100-G peaks demonstrated behavioral loss The animals which exhibited loss were exposed to acceleration profiles of 2000–2500 G-sec

Two of ten animals which were exposed to accelerations of 45–65 G manifested behavioral damage. Acceleration profiles for the damaged animals were 2300–3750 G-sec.

None of the animals which were exposed to less than 45 G demonstrated behavioral evidence of vestibular damage 24 hours after exposure Included in this no-loss group are animals which were exposed to 12 G for periods of 5.5 min and 25 G for over 3.5 min. Profile area measures for the 12 and 25-G animals were 4020 and 5000 G-sec, respectively

Anatomical observations

Gross dissection During development of the dissection procedures several normal animals were examined. Photographs of normal maculae from the utricle and saccule are presented in Figure 4 Maps of the normal saccular and utricular maculae are presented in Figures 5 and 6 Further information about orientation of the maculae may be found in papers by de Burlet and de Haas (1923) Werner (1933) and Spoendlin (1964 1965)

A group of guinea pigs which had been exposed to 300 G on the SFAPS

Fig 4 Photograph of normal maculae from the saccule and utricle The saccular macula, as seen from a lateral view is presented in 6 The otoconia snowdrift which lies over the striola can be detected running through the center of the main part of the macula and hooking to the left in the dorsal lobe Slight damage which occurred during dissection can be detected at the left margin of the dorsal lobe The utricula macula seen from the dorsal medial view is presented in 6 b The striola is represented by the dark crescent which opens to the left, slightly to the right of the center line The otoconia overlying the utricula striola are more sparse than in the surrounding area. Damage which occurred during extirpation can be seen at the lower left hand corner of the macula. Both maculae are from the right ear



b



Fig. 5.

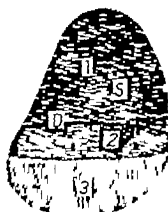


Fig. 6.

Fig. 5. M p of normal saccular macula The dorsal l be is represented by zone 1 transiti nal area is represented by ne 2, nd the main part is represented by zone 3. The stippled re indicates the region of greater otoconi density The dorsal lobe lies in a plane which makes an ngl f bout 30° with the dorsal ntral pl ne The dorsal tip of the dorsal lobe, which is on the left in the m p, is l teral nd the ventral edge f the dorsal lobe is medial. The main part f the saccula ma ul is riented in a plane about 30° off f the dorsal entral plane with the ve tral edge l teral nd the dorsal edge medial

Fig. 6 M p of normal utricular macul The main part of the macula is represented by zone 1 the tran ition area is indicated by zon 2, and the ant rior edge i indic ted by zone 3 The regi n of sparse otoconia er the striola i represented by the tippled area The main part f the macula lies in a plane which m kes an ngl of bout 25 with th l teral-medial plane The l teral edge, which i loc ted at the top f th map, is dorsal and the medial edg i ventral. The anterior edge lies in pla which i bout 30 off th lateral medial plane The transition regi is anterior nd the margin is posterior

were also examined For the 300 G animals, otoconia were observed through out the membranous labyrinth including the ampullae An example of otoconia displacement into an ampulla is presented in Figure 7

Histological obs rvations Figure 8 *a* is a photomicrograph of the left utricular macula (m.u) from animal \ 1 This animal had been exposed to a 100-G peak over a trapezoidal acceleration profile of 30 sec The otoconia from the anterior one-half appear to have been displaced to the posterior part of the macula. Only a few otoconia are present on the macula of the saccule from the same labyrinth (Figure 8 *b*)

The appearance of the left maculae from animal \-3 which was exposed to 50-G peak acceleration over a profile of 55 sec, is shown in Figures 8 *c* and 8 *d* There is an accumulation of otoconia over the posterior end of the

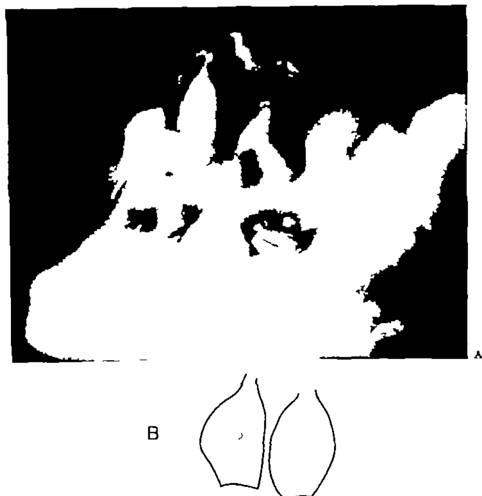


Fig 7 Damage t mpulla f ll wing high linear acceleration. The mpulla of the superior and l teral semicircula canals re depicted i the photograph. Displaced otoconia can be seen i the mpulla f the superior canal which i t the left. The stippled reas in the schematic drawing indicat the location f the displaced otoco ia. Otoconia in this location would account for infrequently bserved positional nystagmus. These mpullae w re t ken from gros dissection practice imal which had been exposed t linea acceleratio of 200 G over tim period f 17 sec in the +G orientation.

left m.u and fewer otoconia present over the remaining portions (Figure 8 c) The macula of the left saccule (m.s) shows a few otoconia evenly distributed except for the posterior margin where none are present (Figure 8 d) Changes in the right labyrinth were practically identical for the m u., but the m.s revealed a complete loss of otoconia.

Animals IX-2 and X-5 were exposed to the same acceleration profile as animal X-3, but in each instance the animal s head slipped caudally and to the left during the exposure The otoconia were practically all removed from the maculae of the left labyrinth of animal X-5, but only slight to

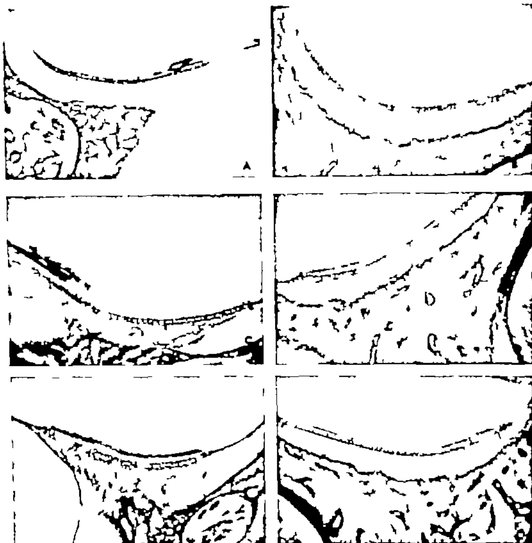


Fig 8 Hist logical bservati for timul durati n peak G imals A Macul f utricl f l ft l byri th f nimal \ 1 showi g loss f otoco l from teri one-half with accumulation o er posteri part 100 G pe k over traperoidal acceleration profil f 30 sec. B Macul f saccul f same labyri th as sh wn l 8 a. There re nly few otoco ia present. C. Macula f utricl f left l byri th f nimal \-3 Otoco ia l ccumulated er posteri end with ly few pri ms remai l g lsewhere 50 G peak accelerati er profil f 85 sec. D Macula f l ft saccul f \ 3 h wing a few rem i i g otoconia pri ms ev ly distributed e cept f posterior margin where none l prese t E M cul f utricl f l ft ear f V-4 showi g light los but some redistribu ti f the toco ia. 25 G pe k over profil f 105 sec F Macul f saccul f same ear show i 8 Central d posterior porti show only few toconia while more are prese t over a teri porti n.

moderate otoconia loss was noted for the maculae of the right labyrinth The findings for IX 2 were not consistent with those from the other animals which had received the same acceleration exposure as only a moderate loss of otoconia appeared along the inferior border of the right m s.

For animal I\-4 the duration of the acceleration profile for 50 G was

TABLE 2 *Otoconia loss ratings for selected animals from experiment I a*

Animal numbe	Ear	Peak G	Rise dwell fall	Post xposure days	Per cent rightings Pre exp	Per cent rightings Post p	Macula f utricle	Cro p	Macula of saccule	Group	I fection
V 1	R	100	5(20)5	15	00	30	5 +	I	−5 +	I	
	L						1 +	II	−4 +	II	
V 2	R	100	5(20)5	15	00	0	−5 +	I	−5 +	I	O.M.
	L						−5 +	I	5 +	I	O.M.
V 3	R	50	5(15)5	15	90	0	−5 +	I	5 +	I	
	L						5 +	I	5 +	II	
V-5	R	25	5(05)5	15	100	100	−1 +	III	−1 +	II	
	I						1 +	III	−2 + P 5 + R	II	
V-6	R	25	5(05)5	15	100	100	−1 +	III	−4 + A 1 + P	II	O.M
	L						−1 +	III	−2 + 4 +	II	O M.
V 5	R	50	5(15)5	7	00	100	2 +	III	−2 +	III	H ad slipped p
	L						−5 +	I	4 +	II	(cla dal) t left.
IX 2	R	50	5(15)5	7	90	100	−2 +	III	2 + 1	III	Head slipped up
	L						−1 +	III	1 +	III	d t left.
IX 1	R	50	5(70)5	7	00	50	5 P 2 + R	II	2 +	III	Head slipped t
	L						5 + P 1 + R	II	−2 +	III	right and d wn
											Hem 1 to CT CMU
IX-8	R	12	5(330)5	7	00	100	−5 + A, 1 + R	II	2 +	III	
	L						1 +	III	+1 +	III	
IX 6	R	25	5(165)5	7	90	100	o − 1 +	III	o − 1 +	III	
	L						o − 1 +	III	2 +	III	

Abbrevi tions: A, terior; P Posterior; L, l ferior S, Superio ; rg., N Chang ; R, R m l de ; Epitheli m; o, Otoco ia; O M Otiti Media; c, Central

increased to 80 sec In this animal the posterior one-half of the right m.u exhibited complete loss of otoconia, and there was a moderate loss from the anterior one half as well as from the m.s. On the left side, the otoconia were missing over the posterior border of the m u., and changes in the m s. were similar to those observed on the right side In this experiment the head of the animal slipped to the right and caudally

Following exposure to 25 G over a profile of 105 sec, the histological observations for animals V 5 and V-6 were similar to those noted previously There was evidence for a redistribution of otoconia without much loss for the utricular maculae as demonstrated for the left m u of animal V-6 (Figure 8 *e*) The macula of each saccule revealed loss of otoconia from the central and posterior areas as shown for the left m.s of V-6 (Figure 8 *f*)

Increase of exposure duration to 205 sec for the 25-G animals (IX-5 IX-6) elicited no major additional changes. As previously the maculae of the saccules showed a greater loss of otoconia than did those of the utricles.

Animals IX 7 and IX-8 received exposure to 12 G over an acceleration profile of 340 sec. Following this exposure animal IX-8 revealed a complete loss of otoconia from the anterior one-half of the right m u and a 2+ change in the m s The left maculae were without much change

In summary stimulus durations of 20 sec at 100 G and 45 sec at 50 G are sufficient to remove most of the otoconia from the maculae of the saccules and utricles. After exposure to 25 G for 95 sec, the maculae of the saccules usually show incomplete removal of otoconia while the maculae of the utricles reveal only slight changes in the distribution of the otoconia. Histological observations for these animals are summarized in Table 2

Experiment I b Linear Acceleration Long-term Recovery

Purpose

The possibility of functional or structural recovery following vestibular damage has been discussed previously (Wittmaack 1909 Hasegawa 1931) The present experiment was designed to explore the possibility of recovery of vestibular capability following exposure to intense linear acceleration In the course of this study behavioral responses of guinea pigs were observed for periods up to 66 days following exposure to various acceleration time histories

Method

Subjects

Thirteen guinea pigs were employed in this study of long-term recovery following exposure to high acceleration The animals were divided into two groups according to date of exposure Nine animals were run in group A and four animals were run in group B



Fig. 9 Position reflex f llowi g high acceleration The position reflex is typically s-sumed f llowing posure t accelerations f 200 t 400 G The animal' head is fl ed nd rotated right. The hind leg co tral teral t the sid of head displacement is ex tended.

Apparatus

The apparatus used in this experiment was the Space Flight Acceleration Profile Simulator which is described in Fxperiment I *a*

Procedure

The procedure in this series of experiments was similar to that described in the previous section. All animals were exposed in the +G orientation as illustrated in Figure 2

Exposure levels and schedule of testing with the righting reflex are given in Table 3 The 13 animals were subjected to peak accelerations of approxi mately 200 300 and 400 G Distribution of the animals among these profiles was as follows 4 animals at 200 G 5 animals at 300 G, and 3 animals at 400 G One animal was retained as a control The acceleration profiles, which were nearly triangular in shape covered temporal durations of about 14 sec for the 200-G peak 17 sec for the 300-G peak and 21 sec for the 400-G peak Immediately after each exposure the subject was examined for nystagmus and general orientation Subsequent behavioral testing with the righting reflex continued for periods up to 66 days. Tests of swimming ability were performed at periods of 2, 30 and 60 days for the animals in group A After completion of behavioral testing all of the animals in this group were transported to the Washington University Medical School where the temporal bones were prepared for histological examination

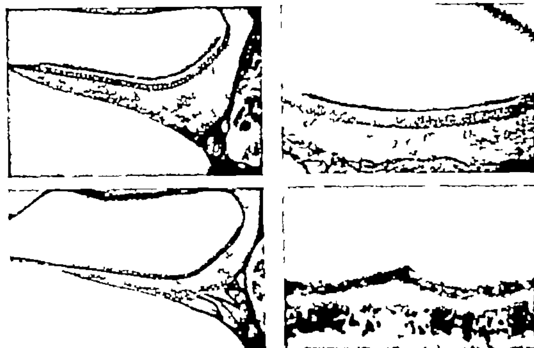


Fig 11 Hist logical bservati n with l g term recovery imal A Macula of the utricle f nonexposed animal showing usual d ity nd distribution of otoconia B. Macul f the saccul of the same a as sh wn i Fig. 11 *a*. The layer of otoconi is compact nd epithell m is without lterati ns. C. Complet l ss of otoc nia from macula f utricl f animal II 3 exposed t 300-G peak nd llowed t surviv for 34 days. D Incomplet loss f otoco ia from nterior e-half f macula of left saccule f II-3 The loss is brupt and epith lial damage i evident. The gelatinous laye is i t ct.

rather erratic after postexposure day 44 The infection may have developed during the recovery period and contributed to the erratic behavior of the recovery functions. Although these data are retained in Table 3, they are viewed with suspicion and were not employed in the plotting of Figure 10

Histological observations

Figure 11 *a* illustrates the appearance of the m u. in a control or non exposed animal The otoconia are in a compact layer and evenly distributed Figure 11 *b* shows the m s. from the same ear at a slightly higher magnifica tion The epithelium of each macula is well preserved

The m u illustrated in Figure 11 *c* shows well-preserved epithelium but a complete loss of otoconia This animal (II-3) had been exposed to a peak acceleration of 300 G and allowed to survive for 34 days. No righting reflexes could be elicited after the exposure The opposite m u revealed a similar complete loss of otoconia The maculae of the saccules showed remaining otoconia near the anterior ends The gelatinous layer remained over the whole surface although most of the otoconia were missing

Figure 11 *d* is a higher power view of the epithelium of the left m.s from animal II-3 The otoconia density is somewhat reduced particularly

TABLE 4 *Otoconia loss ratings for long term recovery animals*

Animal number	Ear	Peak G	Rise dwell fall	Post exposure day	Per cent rightings Pre exp	Post exp	Macula of utricle	Group	Macula of saccule	Group	Infection
II 1	R	200	7(−)5	31	80	80	−5+	I	−1+I 2+R	III	O.M.
	L						−5+	I	o 5+	I	O.M.
II 2	R	210	0.8(−)5	31	100	100	−5A, 3+R	II	5+I 2+R	III	
	L						−5+A 3+R	II	5+I 2+R	III	
II-3	R	310	9( )6.8	31	100	80	−3+	II	−1+I 3+R	II	
	L						−6+P 3+R	II	−2+	III	
II-4	R	295	9(−)7	61	100	80	5+A 3+R	II	5+	I	O.M.
	L						−5+	I	−5+	I	O.M.
II-6	R	205	7.2(−)5.5	61	80	80	5+A 1+R	II	5+	I	O.M.
	L						5+	I	−5+	I	O.M.
II 7	R	410	11(−)8	61	70	0	5+	I	5	I	O.M.
	L						−5+	I	5+	I	O.M.
II-8	R	300	9(−)6.5	31	70	0	−5+	I	3+	II	
	L						5+	I	1+	II	
II 9	R	425	12(−)8	5	100	10	5+	I	5+	I	
	L						5+	I	5+	I	
IV 2	R	200	7(−)5	50	100	70	−3+A, 5+R	II	1+	III	None bout 20 t
	L						−3+A, 5+R	II	3+	II	left during run
IV 3	R	315	9.5(−)8	50	100	0	o −1+	II	5+	I	
	L						−5+	I	1+	II	
IV 4	R	320	10( )7	50	90	100	3+	II	1+	II	
	L						−2+	III	−3+	II	
IV 5	R	430	11(0.1)8	8	90	0					Lal yrI thill O.M
	L										
IV-0	R	430	11(0.1)8	50	90	0	−5+	I	−5+	I	
	L						−5+	I	−5+	I	

to one side of the raised otoconial layer and the epithelium shows some destruction of supporting and sensory cells Similar epithelial changes are not unusual in other specimens.

There is no adequate evidence from this series of 12 animals that any particular reparative process occurs to explain the return of the righting reflexes after exposure However there is a possibility that the gelatinous layer of a macula can be replenished if epithelial damage has not been too severe If this layer in the absence of otoconia, can maintain some func tion of a macula it is important to study it further In the present series of exposures a peak acceleration of about 400 G revealed a complete loss of otoconia and gelatinous layer but the animals that were exposed to a peak acceleration of from 200 to 300 G usually revealed an incomplete loss of otoconia and the presence of an intact gelatinous layer How much if any of the latter can be related to return of righting reflexes during the postexposure period is unknown Results of the histological observations with the long term recovery animals are presented in Table 4

Experiment I c. Linear Acceleration Orientation

Purpose

This experiment was undertaken to determine whether forces acting parallel or perpendicular to the macular surface are most effective in producing damage to the otolithic membrane

Method

Subjects

Nine guinea pigs were exposed to linear acceleration in the +G and +G or −G axes, as illustrated in Figure 2

Apparatus

The Space Flight Acceleration Profile Simulator which is described in the first section was employed in this experiment.

Procedure

The procedure in this series of experiments was similar to that previously described The nine animals were exposed to peak linear accelerations of approximately 300 G Triangular acceleration profiles were employed each profile had a rise time of approximately 0 sec and a fall time of 7 sec The couch was modified to orient the subjects heads in the G or G axes as noted above The exposures for the animals in this experiment are sum marized in Table 5

Results

Behavioral observations

All three animals exposed to acceleration in the +G orientation dem onstrated complete loss of the righting reflex 24 hours following exposure

TABLE 5 *Otoconia loss ratings for orientation experiment animals*

Animal number	ea	Axis and position	Peak G	Rise d ll fall	Post exposure lays	Per cent rightings Post exp. days 1	7	Macula of utricle	Gi p	Macula of saccule	Group	Infection
VIII-1	R	+G	330	9( )7	15	0	50	−5+	I	−1+	II	O.M.
	I							1+	II	−5+	I	O.M
VIII-2	R	+C	335	9.5( )7.5	15	0	0	1+	II	+5+	I	
	L							5+	I	−5+	I	
VIII 3	R	−G	335	9.0( )7	14	0	0	5+	I	5+	I	O.M
	L							5+	I	−5+	I	O.M
VIII 4	R	$+G_y$	330	9.5( )0.5	15	0	90	2+	III	−2+	III	
	L							2+R 1+A	II	−1+	II	
VIII-5	R	+G	300	9( )7	14	100	100	−2+P 3+R	III	1+	II	O.M.
	L							5+	I	1+	II	O.M.
VIII-6	R	+G	300	9( )7	14	100	100	3+	II	−1+	II	
	L							3+	II	1+	II	
X 1	R	−G	300	9( )7	5	0	0	5+	I	5+	I	
	L							5+	I	5+	I	
X 1	R	−G	300	9( )7	5	0	100	−1+	II	1+	II	
	L							2+	III	−1+	III	

whereas two of the five animals exposed to acceleration in the $+G_y$ or $-G_y$ axis exhibited no behavioral loss. By the seventh postexposure day four of the five animals in the G group exhibited completely recovery of the righting reflex, while only one of the three animals in the G group demonstrated even partial behavioral recovery These observations are summarized in Table 5

Histological observations

Exposures in the +G axis at a peak acceleration of 300–335 G with a rise time of 9 sec followed by a fall time of approximately 7 sec resulted in an almost complete loss of otoconia from all maculae Examples of the histological results following these exposures are presented for the right m u from animal X-4 in Figure 12 *a* and the right m.s from animal X-4 in Figure 12 *b* In rare instances there were a few otoconia remaining on the macular surfaces, but these had probably been dislodged during the exposure and had settled back into position

Exposures in the G axis, with the cephalocaudal head axis perpendicular to the plane of rotation, to acceleration profiles similar to those for the G animals gave rather conflicting results Of the two animals for which otoconia were entirely lacking from all maculae one apparently had a marked otitis media that could have been present at the time of exposure, and the other had severe autolytic changes in the inner ear because it had died several hours before the immersion of the temporal bones in fixative The two remaining animals, which received exposure in the G axis with the cephalocaudal head axis perpendicular to the plane of rotation exhibited moderate to severe otoconia loss. Figure 12 c illustrates the moderate otoconia loss which extended over the entire surface of the left m u from animal X 1 The right m u. from this animal, which showed complete loss of otoconia is illustrated by Figure 12 *d* The left m s. of animal X 1 was little altered but the right m.s was stripped of otoconia The right m u. and m.s. from animal VIII-4 showed a slight loss of otoconia while changes in the left m u and m.s resembled those seen in the right labyrinth of animal X 1 These findings are interesting because animal X 1 was exposed in the −G orientation, whereas animal VIII-4 was exposed in the +G orientation

Exposures in the G axis with the cephalocaudal head axis parallel to the plane of rotation resulted in an almost complete loss of otoconia from the left m.s. and m u of animal VIII-5 The right macula revealed similar changes, but less marked, over the posterior one-half of the m.u Following a similar acceleration exposure, animal VIII-6 demonstrated a similar pattern of otoconia loss for both the right and left ears The maculae of the saccules showed only a few scattered otoconia on their surfaces (Figure 12 *e*) while the maculae of the utricles revealed an accumulation of otoconia in the central regions with a total loss over the posterior areas (Figure 12 *f*)

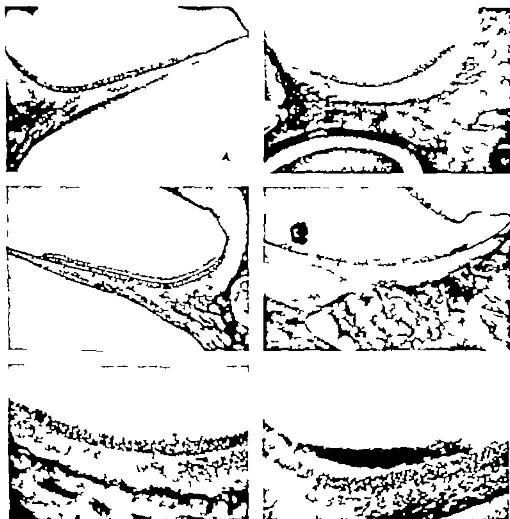


Fig 12. Hist logical observations with orientation nimals A. Macula f right utricl f animal X-4 showing complet loss f otoconia. Exposure in −G axis to 200-G peak acceleration with rise time f 9 sec nd fall time f 7 sec. B. Macula of right saccul f sam ea as Fig. 12 a howing only ery few remai i g otoconia prisms. C. Macul f left utricl f animal X 1 howing moderat but consistent loss f otoconia over the entire surface Exposure was in the −G axi t 200-G peak acceleratio with rise of 9 sec nd f ll f 7 sec. D Macula of right tricl f same nimal as Fig 12 showi g marked loss f otoco i The otoconia of the macula of the left saccul howed practically no loss, whil the right m.s. wa stripped of otoconia. E. Right m.s. f nimal VIII-6 showi g an lmost complet loss f otoconia. F Macul of right tricle of same ear as Fig 12 showing accum lation f otoconia in centr l ne-third with total loss over posterior ne-third. The l ft m.s showed simila cha ges.

In conclusion, exposures in the +G axis produced the most consistent damage as evidenced by severe loss of otoconia from all maculae Exposures in the G axis with the cephalocaudal head axis perpendicular to the plane of rotation produced changes which were greater in one ear than the other

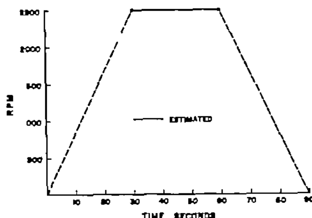


Fig. 13 Exposure profil for ial rotatio The ordinat indicates revolutions pe mi at d the bscissa represe t tim from stim lu onset. In this case the posure profile had rise, dwell, nd decay times f 30 sec Rotati rat duri g rise d dec y tim wa estim ted.

with the cephalocaudal head axis parallel to the plane of rotation, the right and left maculae were affected similarly These observations are given in Table 5

Experiment I d Linear Acceleration Combination With High Axial Rotation

Purpose

Investigation into the effects of axial rotation was undertaken to duplicate an earlier series of studies by Wittmaack (1909) It is hypothesized that the major damaging effect of axial rotation is caused by the linear accelera tion component for the acceleration profiles used in the present study However it is possible that damage to the cristae reported by Wittmaack and his students was, in part a function of the angular acceleration component Unfortunately the distance between the maculae and the axis of rotation which Wittmaack employed is not entirely clear For the present series of investigations, rotation around the cephalocaudal axis was chosen

Method

Subjects

Five guinea pigs were rotated at 1800 or 2500 rpm to investigate the ef fects of axial rotation on the vestibular apparatus.

Apparatus

An aluminum tube which was 25 cm long and had an inside diameter of 6 cm restrained the animal during exposure A tooth bar covered with dental impression compound provided head restraint and a Hardinge metal lathe was used to spin the animals.

TABLE 6 *Results of axial rotation study*

Animal	Peak rpm	Acceleration profile			Righting loss 2 hrs	Gross dissection
		Rise	Dwell	Fall		
AR 1	2500	30	30	30	–	–
AR 2	2500	30	30	30	+	OM
AR 3	1800	30	30	30	–	N/O H
AR-4	2500	30	30	30		N/O H

+ Observable indicated damage; – Observed, not indicated damage; H, Retained for histological examination; N/O, Not observed; OM, Otitis media.

Procedure

Before each experiment the guinea pigs were subjected to tests of the righting reflex, as indicated under Experiment I a The animal was then placed in the aluminum tube and the head was fixed by the tooth bar which was covered with warm dental impression compound The tube was attached to the lathe, and the animal was rotated for 90 sec The profile for this acceleration, which is presented in Figure 13 indicates a 30-sec rise time to a peak rotation rate of 2500 rpm maintained for 30 sec and succeeded by a 30-sec fall time The maximum angular acceleration with this exposure is about 500 /sec² A 90-sec acceleration profile was also employed for the 1800 rpm peak rotation exposure Rotation rate during rise and fall time was estimated If the maculae are assumed to be one cm from the axis of rotation peak linear acceleration at 2500 rpm is approximately 70 G and for 1800 rpm peak acceleration is approximately 36 G

Results

Behavioral observations

Following exposure to 2500 rpm two of three guinea pigs demonstrated behavioral loss, as indicated in Table 6 The one animal exposed to 1800 rpm did not exhibit loss of the righting reflex.

Gross dissection observations

Gross dissection of the labyrinth was performed on one of the 2500-rpm animals. In this animal most of the utricular otoconia were in the normal position. However a fine scattering of otoconia was observed on the utricular walls opposite the maculae and into the ampullae of the superior and lateral semicircular canals.

Saccular otoconia were considerably displaced in both ears In the right ear the otoconia were completely removed from the saccular macula and were lying in a clump against the anterior lateral wall of the utricle. For the left ear the posterio end of the macula was not disturbed but otoconia from the anterior end of the macula were displaced in the posterior ventral

direction The gross anatomical picture for the saccule of the left ear was somewhat unusual in that the otoconia prisms retained their spatial relationship to one another and appeared to be folded as a group It would appear that the otoconia and the gelatinous basal layer were displaced together rather than the otoconia being thrown from the gelatinous layer

Histological observations

The two guinea pigs exposed to axial rotation and for which histological studies were made showed considerable displacement of otoconia The m.s of the left ear of AR-4 revealed a stripping of the otoconia with the gelatinous layer intact (Figure 14 *a*) and rupture of the membrane of the saccule The m.u of the same ear showed an incomplete loss of otoconia particularly over the posterior half This was also true of the opposite m.u., but the right m.s showed intact otoconia with relatively slight loss AR-4 was subjected to 2500 rpm

Animal AR-3 which was rotated at 1800 rpm showed otoconia adherent to the cupula of the lateral canal crista (Figure 14 *b*) The right m.s showed some moderate loss of otoconia with collapse and rupture of the saccular membrane Figure 14 *c* shows the right m.u with otoconia loosened from their usual compact positions but in addition the posterior portion of the macula had been pulled loose and reflected in the anterior direction.

Experiment II Vibration

Purpose

The possibility of vestibular damage as a result of exposure to low frequency vibration has been suggested (Riopelle Hines, and Lawrence 1958) In the present investigation guinea pigs were exposed to vibrations of 1–7 G at 6–20 Hz for periods of 4–8 hours to examine the possibility of vestibular damage

Method

Subjects

Twenty-one guinea pigs were subjected to moderate low frequency sinusoidal vibration for periods up to 8 hours The animals were divided into seven groups, A through G according to exposure conditions

Apparatus

The vibration stimulus was obtained with an MB Electronics Model S-3 vibration exciter The restraint device consisted of three 21-cm troughs attached to a 30- by 30-cm plate fastened to the head of the vibration exciter

Acceleration magnitude was calculated using the result of stroboscopic determination of displacement of the base plate Examination of the output of an accelerometer mounted on the plate revealed little evidence of waveform distortion at the frequencies used.



Fig 14. Histological observations of animals exposed to ... lal rotation. A. M cul ... f left saccul of AR-4 showing strippi g f the otoconi ... nd gelatinous laye from its surface. This ... imal had been subjected t 2500 rpm with ... n acceleratio profile of 30 sec rise 30 sec dw ll, ... d 30 sec f ll. The membrane of the saccul was ruptured. The otoconia were intact on the right m.s. Both m.u. showed incomplet loss f otoconia ver the posterio one-half. B. Segment of gel tinous lay ... d otoconi ... dherent t th left l teral canal cupul. Animal AR-3 w ... subjected t 1800 rpm with the sam acceleratl n profile ... AR-4. There was ... l ... moderat loss of otoconia from m.s. and m.u. f each ear. C. Macula of the right utricl ... f the same ... imal as in Fig 14 b sh wi g detachment of the posteri one-half f the macula ... nd its reflecti ... teriorl. Otoconia is dispersed with som loss.

Procedure

Before exposure the animals were tested with the righting reflex, and only those animals which were normal according to the criteria indicated in Experiment I *a* were used. The chosen animals were placed in whole body plaster casts and taped in the troughs. During each run the animals were examined periodically with the stroboscope to assure that the restraint remained secure. These observations indicated that the whole body cast provided an excellent restraint device. It is probable that the actual stimulus received by the maculae was very close to that recorded by the accelerometer mounted on the plate.

The animals were oriented in three positions with respect to the direction of vibration by rotation around the cephalocaudal axis. When the head

TABLE 7 *Behavioral and gross dissection observations for vibration studies*

Animal	Head tilt Degrees	G (Peak)	Exposure Frequency	Exposure Duration	Righting loss 1 hr	Righting loss 24 hrs	Gross dissection
A 1	0	1	8	4 hrs	−	−	N/O
A 2	45	1	8	4 hrs	−	−	N/O
A 3	90	1	8	4 hrs	+	+	+
B-1	90	1.25	8	4 hrs	−	−	N/O
B-2	90	1.25	8	4 hrs	−	−	N/O
B-3	90	1.25	8	4 hrs	+	−	+
C 1	90	1	6	8 hrs	−	−	N/O
C 2	90	1	6	8 hrs	−	−	N/O
C-3	90	1	6	8 hrs	−	−	N/O
D-1	90	7	20	4 hrs	−	−	OM
D-2	90	7	20	4 hrs	Died in apparatus		
D-3	90	7	20	4 hrs	Died in apparatus		
E 1	0	3	11	6 hrs	−	−	−
E 2	0	3	11	6 hrs	−	−	−
E-3	90	3	11	6 hrs	+	−	−
F 1	0	2	8	6 hrs	−	−	N/O H
F 2	90	2	8	6 hrs	−	−	N/O H
F-3	90	2	8	6 hrs	−	−	+
G-1	0	1	6	6 hrs	−	−	N/O H
G-2	90	1	6	6 hrs	+	−	N/O H
G-3	90	1	6	6 hrs	+	−	−

+ Observations indicate damage. − Observation does not indicate damage. H, Retained for histological examination. N/O, Not observed. OM, Otitis media.
Head tilt of 0 degrees: vibration in the G axis; head tilt of 90 degrees: vibration in the G axis (see text).

tilt was 0 the acceleration vector was oriented in the G axis, as depicted in Figure 2 for 90 head tilt, vibration was in the G_y axis. The third head tilt position was 45

Following exposure the animals were again tested with the righting reflex at periods of 1 hour 24 hours, and in one case 7 days. After completion of final behavioral testing, four of the animals from Groups F and G were saved for histological examination. Of the remaining animals, seven were examined by gross dissection.

Results

Behavioral observations

A summary of the vibration studies, including the exposures and the results of observations with the righting reflex and gross dissection is



Fig. 14. Damag f llowi g pos re t ibration. The photograph depict damaged saccul macul from imal F-3 which had been exposed t ibration at 1.25 G (peak) nd 8 H for 4 hours. The stippled re on the schematic drawi g below the photograph indicates the regi of otoconia displacem t. D mag occurred long the entral edg f the dorsal l be

presented in Table 7 Behavioral loss was exhibited by 5 of the 21 animals exposed to vibration. Only those animals which were exposed to accelera tion in the G axis demonstrated behavioral loss according to the criteria presented in Experiment I a Of the five animals which demonstrated behavioral loss at 1 hour postexposure only one animal, A-3 continued to exhibit los at 24 hours. This animal was unable to perform the righting reflex just before its termination at 7 days.

Gross dissection observations

Gross dissection of the temporal bones was performed on 7 of the 21 animals exposed to vibration Included in these seven were four of the five animals which demonstrated behavioral loss the fifth behavioral loss animal was transported to Washington University Medical School for histological processing Evidence of structural damage was observed in three of the seven animals subjected to gross dissection Two of the three animals which showed structural damage had manifested behavioral loss before termina tion In one animal A-3 this damage consisted of displacement of otoconia around the margins of the maculae For animal B-3 structural changes con sisted of displacement of otoconia in the m u of the left ear Direction of otoconia displacement was from the lateral-dorsal area toward the medial ventral area For animal F-3 damage was observed in the m.s. of the right ear In this animal a small clump of otoconia was observed just beyond the anterior margin of the macula An illustration of the damage observed in animal F-3 is presented in Figure 15

In most of the temporal bones examined from both exposed and control animals, some scattering of otoconia around the margins of the maculae was observed Whether this scattering is a function of the dissection procedure or is a normal condition is not known

Histological observations

The histological findings for the four guinea pigs which were subjected to vibration revealed a minimum loss of otoconia, particularly from the m.u The posterior one-half of the left m u from animal F 1 is shown in Figure 16 *a* This animal had been exposed to vibration at 2 G, 8 Hz for 6 hours in the G axis The supporting and sensory cells appear altered and there is loss or displacement of some of the otoconia The central and anterior parts of the same macula exhibited little alteration as illustrated in Figure 16 *b* The m.s from the left ear demonstrated only slight changes in the distribution of the otoconia Findings were similar for the right ear except for a greater loss of otoconia from the m.s. Animal F 2, which had received the same vibration exposure as F 1 in the G axis, showed similar changes in both m.s and m u

The left m u from animal G-1 which was exposed to vibration at 1 G 6 Hz for 6 hours in the G axis, is presented in Figure 16 *c* As this figure

Fig. 16 Hi tological bsers ti nimal exposed t vibratio A Posterior e-half f macula f the l ft utricl f imal F 1 showi g som dispersi d loss f toconia. Th imal w posed t vibrati t 2 G (pe k) 8 Hz for 6 hours i the G a is B. The central nd terior porti on f the sam m.u as show i Fig 16 *a* bowing i tact l yer f otoconia Througho t thi m.u. there wa evidence f supporti g cell and sensory cell damage The macul f the saccules showed light dispersion which w great f the right tha left ea C. Macula f th left utricl f im l G-1 showing disruption f otoconia Thi expos re was 1 G (peak) t 6 Hz f 6 hours i the G axis. The pposit m.u. w imila whil the loss of toconi was greater from each m.s.


A



Table 8 *Otoconia loss ratings for vibration study animals*

Animal number		Head tilt (degrees)	Exposure G (peak) frequency duration	Righting loss (1 hr) (24 hrs)	Macula of utricle	Macula of saccule
	R		2 G		o-neg	o-3+
F 1		0	8 Hz			
	L		6 hrs		o-1+R, 2+P	o-1+
	R		2 G		o-3+P 1+R	o-2+
F 2		90	8 Hz			
	L		6 hrs		o-1+	o-1+
	R		1 G		-1+	o-2+
G 1		0	6 Hz			
	L		6 hrs		-2+	-1+
	R		1 G	+	o-1+	o-2+
G-2		90	6 Hz			
	L		6 hrs		-neg	o-2+

All animals were in damage group III

indicates, there is loss from the anterior and central areas similar findings were obtained from the right m u The m s. for each ear revealed con siderable loss of otoconia over the entire surface without disruption of the membrane layer Animal G-2 for which the exposure was the same as with G 1 except that the head tilt was 90 exhibited a similar pattern of otoconia loss.

In conclusion the four ears of the two guinea pigs exposed to an 8 Hz vibration at 2 G revealed similar changes in otoconia loss and displacement The posterior one-half of the m u apparently is more vulnerable to the exposures. The m.s. revealed reduced otoconia and some changes in their distribution No differences in results were discernible between the positions of the head during exposure The four ears of the guinea pigs exposed to 6 Hz at 1 G revealed a loss of otoconia from the m u of the guinea pig exposed with 0 head tilt which was not apparent in the specimen which was in the 90 position No differences were apparent for the otoconial loss from the m.s. in the four ears. These histological observations are sum marized in Table 8

Experiment III Loud Sound

Purpose

Previous investigation has indicated that structural damage takes place in the vestibular area in man and lower animals with exposure to intense

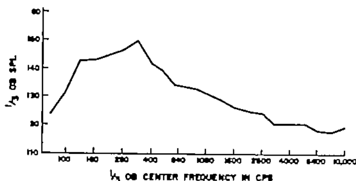


Fig 17 Frequency-i ten ity pectrum for noise t subject ears. The ordi t indicates sound pressure level (SPL) in dB re 0.0002 microba nd th bscissa represe t oct band (OB) center frequency in Hz.

auditory stimulation (Rüedi and Furrer 1946 McCabe and Lawrence 1958 Albernaz, Covell and Eldredge 1959) Also, some preliminary observations indicate that intense auditory stimulation may produce transient loss of equilibrium-related reflexes during stimulation (Ades, 1953) The present study was undertaken to replicate the previous work and to attempt the correlation of behavioral loss with structural damage

Method

Subjects

Ten guinea pigs were exposed to wide-band noise at intensities of 140 148, and 154 dB SPL.

Apparatus

The wide-band acoustic siren described by Cole, Powell, Oestreicher and von Gierke (1963) was used to provide the auditory stimulus. Overall sound intensities at the location of the experimental animals were 140 148, and 154 dB re 0.0002 microbar The spectrum of peak intensity versus frequency is presented in Figure 17

Procedure

All of the animals used in this experiment gave behavioral results within the normal range before exposure Four animals were exposed to 140 dB, 4 animals to 148 dB and 2 animals to 154 dB SPL. Duration of all exposures was 20 min. After exposure, tests of the righting reflex, pinna reflex, and swimming ability were performed. One animal was retained for histological examination, and the temporal bones from two animals were studied by gross dissection.

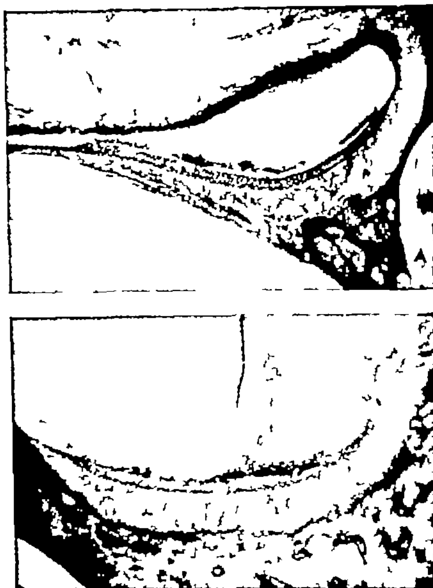


Fig 18. Hist logical bserv tions animal xposed t loud sound A. Macula f th right ut icl f a guinea pig exposed t wide-band n ise t 154 dB SPL for 20 mi utes. The toco i ppe t be l mped i t gro ps f varyi g izes with some loss. Th utricula membra w ruptured (ot shown) B. M cul f the right saccul f th same bow i Fig 18 showi g a central mass f otoconi nd few elsewhere There i rupture nd collapse f th saccula membrane Th macul e f th left ear were simil rly d m ged by the i tense sou d xposure

Results

None of the animals exposed to loud sound gave evidence of behavioral loss in terms of the righting reflex and swimming ability All of the subjects exhibited complete loss of the pinna reflex.

Gross dissection revealed no changes in the vestibular labyrinth the saccular and utricular membranes did not appear to be collapsed

The temporal bones from the one animal which was exposed to 1о4 dB SPL and was retained for histological examination revealed almost complete destruction of the organ of Corti for all turns of each cochlea There wa some collapse of the saccular membrane of both ears and evidence f r rupture of the right utricle Figure 18 a illustrates the appearance of the otoconia of the right m u The otoconia appear to be in small aggregations with some loss. The findings were similar for the left m u Figure 18 b shows the collapsed and ruptured saccular membrane from the right ear with the otoconial layer folded over onto itself at one point Finding were similar for the m.s of the opposite ear



Fig 18. Hi t l peal observations n nimal exposed t loud sound A M cul f the ight t i l f gui ea pig xposed t wid ba d noise t 154 dB SPL for 20 min tes. The otoco ppe t be l mped i t groups f v rying izes with some loss. The tricula m mbra w ruptured (t shown) B. M cul f the right saccul f the same sh wa Fig. 18 a howi g central mass f otoconi nd few lsewhere There i rupture nd coll pse of the saccul membra The macul f the left r were simila l dam ged by the intense sound exposure

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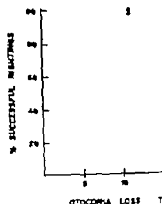


Fig 10 Scatter diagram rel ting righting refl ca ordinate indicates per cent successful rightings at represents the sum f histological rati gs f to moment correlation coeffi ient calcul ted from th

that the threshold for structural damag the threshold for behavioral damage the e following exposure to 12 and 25 G sustaine has been somewhat surprising Future exper development of behavioral techniques sen t structural damage associated with this l w tion.

Relationship to Previous ...

Linear acceleration relationship between exposur duration and peak G

Behavioral observations The results of our investi ati n wh e r su marized in Figure 3, indicate that the threshold for l ss f th righting reflex is about 50 G applied for at least 25 sec The shape of the threshold curve is approximately that which might be expected if the ot l thic apparatus behaves like a mass-spring system

Wittmaack (1909) performed qualitative observations of equilibrium disturbances for his animals, which had been exposed to centrifugation at 2000 rpm around the cephalocaudal axis He noted the rolling and general unsteadiness which has been previously described for the animals in the present study However as we have noted, these effects are transient, usually disappearing within 24 hours after exposure

Hasegawa (1931) reported that behavioral loss was not produced by peak accelerations of 250 G applied over a triangular acceleration profile of 10 sec, whereas increasing the peak G to 290 did elicit behavioral loss. The differences in threshold for behavioral loss observed by Hasegawa and the present study indicate the necessity for examining the techniques to assess behavioral loss in the two studies Hasegawa used

4 Parker

relation to various body positions and the progressive reaction, whereas the righting reflex and swimming ability were used in the present experiment However it would seem that performance of the progressive reaction for vertical linear movement would require the same information from the vestibular system as performance of the righting reflex. As previously noted, we found that behavioral loss was somewhat difficult to judge employing the progressive reaction It appears that the righting reflex and swimming ability provide a more sensitive indicator of vestibular damage than do tests of eye rolling and the progressive reaction

Anatomical observations Dislocation of otoconia has been observed in the present investigation following exposures as low as 12 G for 340 sec. Hasegawa has stated that the threshold for anatomical loss appears to be lower than the threshold for behavioral loss he found displacement of otoconia following exposures to 250 G for 10 sec without simultaneous behavioral loss Unfortunately Hasegawa s observations did not extend below 250 G

Margaria Gualtierotti, and Spinelli (1958) have reported that the threshold for otoconia detachment in small fish and frogs is about 150 G applied for 1 min This stimulus is considerably more intense than the minimum amplitude which would elicit evidence of vestibular damage in the present experiment

Spoendlin Schuknecht and Graybiel (1965) reported that no structural damage could be observed in the vestibular area after exposures of squirrel monkeys to 10 9 G for periods up to 10 min The light microscopy photomicrograph of the macula from an animal which was exposed to 10 9 G for 1 min certainly supports this conclusion However the two animals which were exposed to 10 9 G for longer periods (600 and 300 sec) died while in the centrifuge It is possible that the authors might have been cautious in reporting slight changes in these animals because of autolytic difficulties

De Vries (1950) reported loss of a saccular otolith in 1 of 20 fish subjected to an 11 G acceleration probably for more than 1 min.

Differences between the results of the present investigation and those from previous studies cannot be easily elucidated at present In the earlier studies by Wittmaack and Hasegawa the criterion for damage was disturbance of the entire otolithic membrane, whereas in the present study damage was recorded if the otoconia were disturbed even though the gelatinous layer remained intact. Perhaps similar disturbances of the otoconia have been observed by previous investigators, but they have been unwilling to make any statements because of inconsistency in their material This leads to question regarding possible histological artifacts which will be discussed in a subsequent section

Linear acceleration long-term recovery

Behavioral observations The results which we have presented support the hypothesis that functional recovery may take place for orientation re-

flexes normally mediated by the vestibular apparatus following severe behavioral disturbance This recovery as determined by observations of the righting reflex and swimming ability took place from 1 to 40 days following exposures to accelerations of 200–300 G for periods of 14–18 sec On the basis of the behavioral data it is not possible to state whether the recovery locus is central or peripheral

The peripheral recovery hypothesis was first suggested by Wittmaack (1909) He noted that equilibrium disturbances generally disappeared within 24 hours following one exposure to centrifugation. Recovery time increased to as much as eight days following five exposures to centrifugation. Employing quantitative techniques, Hasegawa (1931) was unable to find any behavioral evidence of recovery for up to 110 days following acceleration exposure However Hasegawa s acceleration of 290 G is close to the level of irreversible behavioral loss as determined in the present investigation Also, as has been previously noted behavioral loss may be difficult to judge employing Hasegawa s techniques.

Fernandez has suggested that the recovery which we have observed may be central rather than peripheral This central recovery hypothesis could be dependent on (1) repair of damage to the neurons or vascular supply of the vestibular pathways, or (2) central compensation such as learning Regarding the first possibility serial sections through the vestibular nuclei of the animals in the present series have not revealed adequate evidence of neuronal degeneration or hemorrhage The possibility of learning is less easy to eliminate. However one might ask why learning should take place in the animals which had been exposed to 200 and 300 G but not for the 400-G animals.

Anatomical observations There is no evidence from the present investigation to support the hypothesis that the otoconia are reformed during the recovery period Wittmaack states that he was able to observe a suggestion of otolithic membrane reformation with careful study of his slides. Conversely Hasegawa saw no evidence of otolithic membrane reformation even after 110 days of recovery

The results of the present investigation however do not preclude in some instances the possibility of repair of the gelatinous layer While the composition of the gelatinous layer has not been completely determined, it may be suggested that movement of this layer alone could produce some stimulation of the macular hair cells. If reformation of the gelatinous layer was sufficiently complete it might then mediate recovery of the behavioral orientation reflexes.

Linear acceleration orientation

Observations by von Holst (1950) on fish and Schöne on humans (1962, 1964) have demonstrated that accelerations acting parallel to the maculae which produce shearing forces, comprise the effective stimulus for these organs. In view of these observations, the question has been raised as to

whether acceleration acting parallel or perpendicular to the macular surface would be most effective as a damage producing stimulus

Major consideration was given to the utricles in planning this experiment While the utricles are known to respond to linear acceleration and are involved in the equilibrium reflexes there has been considerable discussion about the function of the saccule during the past five decades. Among others, McNally and Tait (1925) and Versteegh (1927) removed the saccule or cut the saccular nerve and were unable to detect any disturbance of equilibrium function as a result of the saccular ablation Recently Jongkees (1950) working with rabbits, and Walsh (1960) with humans, have been able to detect saccular responses to linear acceleration The weight of experimental evidence however indicates that the utricle is the major mediator of the vestibular equilibrium responses, and that the saccule, while responsive to linear acceleration is of secondary importance

When a guinea pig is oriented in the G position in the SFAPS the forces acting on the utricular maculae are nearly parallel to the macular surface whereas *in the G orientation acceleration is almost perpendicular to the* macular surface Both behavioral and histological data in the present investigation indicate that acceleration acting perpendicular to the macular surface is most effective in producing displacement of otoconia Unfortunately insufficient studies were performed to obtain statistical proof of this hypothesis

Moreover the only animals that demonstrated behavioral loss in the vibration experiment were those placed in the G_y orientation As this problem could conceivably be of interest in the design of emergency reentry vehicles for use in space exploration further investigations should be performed

Linear acceleration axial rotation

Wittmaack (1909) noted that centrifugation resulted in throwing off of the entire otolithic membrane for his animals. The otolithic membranes were described as being rolled up in balls and lying in the corners of the utricle and saccule Hasegawa s observations (1931) were similar to W maack s. The vestibular damage observed in the present investigatio not seem to have been as severe as that described by Wittmaack. P ur estimate of a distance of one cm from the axis of rotation to the n for Wittmaack s study is in error

Vibration

Only slight evidence of vestibular damage following exp tion has been seen with behavioral or anatomical observation study The only animal which demonstrated loss of the ri hours after exposure showed hemorrhage in the vestibular

section revealed evidence of mild otoconia displacement in three animals.

Riopelle Hines, and Lawrence (1958) reported that monkeys which were exposed to 2.6 G (peak) sinusoidal vibration at 10 Hz for 8 hours demonstrated no behavioral loss in terms of jumping ability and pattern discrimination tests. Histological observation of a group of monkeys which received similar vibration exposure revealed slight evidence of otolithic membrane displacement for three of six animals.

Difficulties in determining the vibration amplitude actually received by the maculae complicates comparison of the results from Riopelle *et al* and those from the present study For both investigations, vibration amplitude was determined at the support for the restraint device It is almost certain that the vibration amplitude at the head was not the same as at the support The restraint device used in the present investigation, consisting of a whole-body cast probably provided a more rigid support for the head than the chair used by Riopelle *et al* Therefore for the same displacement, the amplitude of head vibration probably was greater in the present study than in the one by Riopelle *et al*

Results from the two investigations do not appear very different. While the righting reflex is probably a better test of vestibular capability than jumping ability neither behavioral test gave much evidence of damage On the anatomical side moderate evidence of disturbance to the maculae was seen in each study

Loud sound

Experiments on the effects of exposure to loud sound clearly reveal that vestibular disturbance can be produced by an auditory input (Ades, 1953 Rüedi and Furrer 1947 McCabe and Lawrence 1958 Albernaz, Covell and Eldredge, 1959) Experimentation on the possibility of producing vestibular damage by exposure to loud sound has been reported by McCabe and Lawrence and Albernaz *et al* These authors report rupture of the membranous partition and saccule following exposure to noise or pure tone at intensities of 140–153 dB SPL. Rüedi and Furrer report damage in the vestibule and semicircular canals following exposure of a guinea pig to an explosion

The results of our investigations with the righting reflex and swimming ability indicate no behavioral los following exposure to loud sound. Ades has reported transient interruption of the righting reflex for one cat exposed to 960 Hz at 140 dB SPL. A second cat exposed to loud sound irregularly varied over the range of 200–2000 Hz, f iled to show any postexposure deficiencies in postural reflexes. The results of the present investigation indicate th t the results given by the cat which demonstrated transi nt behavioral l s are the exception rather than the rule

Althougt no evidence of membrane collapse was seen with gross dissection examination of serial sections of one specimen revealed s me evidence of utricu ar and saccular membrane collapse as well as severe damage in

the cochlea These findings are in line with the results of the previous studies.

The animals in the present study were not anesthetized during the sound exposure It is likely that the middle ear reflex attenuates the sound transmission to the inner ear Perhaps greater evidence of damage could be found with anesthetized animals Anesthesia was not employed because it would have prohibited or confounded behavioral testing immediately after exposure In view of the limited evidence of damage found in these studies it seems unlikely that the use of anesthesia would produce markedly different results.

Positional Nystagmus

Nystagmus was exhibited by animals which were placed in particular positions following ... high acceleration on the SFAPS Anatomical observations (Fig ... support the hypothesis that this positional nystagmus is ... mulation of the crista by displaced otoconia thrown into the ... observations and conclusions are in agreement with ... awa (1931) and de Kleyn and Versteegh (1933)

In 1921 ... ical observations of nystagmus which occurred wh... d in certain critical positions Citron and Hallpike ... tudies which indicated that this positional nystagmu... nign paroxysmal type is dependent on lesions ... These authors stated that a "significant proporti... vidence of labyrinthine trauma. During developm... n procedures for the present study some scattering ... quently seen around the margins of the maculae ... e blows or impacts, such as those associated with aut... falling against bathtubs, otoconia may be thrown int... h displacements provide one possible explanation for the ... benign paroxysmal positional nystagmus

Approaching the ... from a clinical viewpoint, Schuknecht (1962) has proposed the ... lisl cation hypothesis to account for benign paroxysmal positional nystagmus According to Schuknecht's view the otoconia could be ... result of degenerative changes in the maculae, perhaps as a result of occlusion of the anterior vestibular artery as well as by displacement resulting from acceleration

A recent experiment which indicates that a true nystagmus may be elicited from the statolithic organs alone indicates caution in applying the otoconia displacement hypothesis universally Benson and Bodin (1966) rotated men at a constant rate around the cephalocaudal axis and were able to record nystagmic eye movements These results certainly allow the hypothesis that an abnormal pattern of nerve firing from the maculae alone may produce nystagmus and vertigo

Comments on the Righting Reflex

The righting reflex has frequently been used for determining the functional condition of the vestibular apparatus. Rademaker (1935) performed extensive studies using this technique on cats. Following extirpation of both labyrinths, Rademaker noted that there was a complete loss of the righting reflex that the animals fell to the floor motionless like "a sack of potatoes" This is certainly an apt description for the results of observations with guinea pigs which had been exposed to 400 G in our studies.

Hüttepohl (1936) has performed extensive observations of the righting reflex with frogs. He noted that the righting reflex is composed of four distinct components (1) turning of the head and upper extremities, (2) turning of the torso and hind quarters, (3) splaying of the extremities in a braking action, and (4) flexing of the extremities to cushion the landing Although quantitative observations of these four stages were not performed in the present study it was noted that animals which were partly recovered, or those which had been exposed to lower stimulus intensities were frequently able to perform the first components of the righting reflex. When the complete righting reflex was not performed, the failure was generally attributable to high latency of the initial turning or absence of the braking movement

Changes in the time relations of the righting reflex with partially recovered animals allows an interesting speculation As noted previously it is possible for centrifugation to completely remove the otoconia with the gelatinous layer from the macular surface While there is no evidence for reformation of otoconia in the postexposure period, the possibility of resecretion of the gelatinous layer has not been excluded Perhaps the hair cells of the maculae can be stimulated by movement of the gelatinous layer in absence of otoconia. If this were so, however because of the differences in the mass of the gelatinous membrane as opposed to the otoconia, it would be expected that the time constant for the system response would be considerably lengthened and the sensitivity reduced. Such timing changes in the otolithic system could well lead to changes in the latency of various components of the righting reflex

Hüttepohl also investigated righting ability as a function of the height of the drop Initially we planned to obtain similar functions in the present experiment. However two factors led to abandonment of these plans. First the animals which demonstrated at least partial behavioral los appeared to fatigue after landing repeatedly on the back or side. Therefore the number of drops each day was limited to ten. With so small a number of observati ns, it was not possible to obtain the distribution of righting ability as a function of height Second, the optimal height of dropping varied with the same animal during the course of the experiment. For guinea pigs the optimal height of dropping varied with the animal's weight. Small (200–250 g) guinea pigs were used for the xposures. However during the

postexposure period the animals gained weight rapidly This was particularly true of the long-term recovery animals. Therefore the height of dropping distribution would not be comparable for the same animals from week to week

Extraneous variables such as "freezing" were controlled by the manner in which the animals were dropped The experimenter held the animal at the desired height by the legs and nose With experience it was possible to judge when the animal was in a relaxed state and to release it at that time If the animal was dropped while it was still struggling (most animals resisted being placed in an inverted position) its ability to perform the righting reflex was reduced. Therefore it was thought that slight variations in the manner in which the animals were held were compensated by the ability to judge the animal s state at the time of the drop

It has been stated that visual input has no influence on the righting reflex This appeared to be true for animals that demonstrated complete behavioral loss. However for those guinea pigs which demonstrated partial behavioral loss, restoration of vision appeared to improve the ability to perform the reflex. Unfortunately quantitative investigations were not performed on this subject

Comments Regarding Angular Acceleration

The maximum angular accelerations received by the guinea pigs in this study were approximately 580 /sec² for the SFAPS exposures and 500 /sec² in the axial rotation studies The latter figure is only an estimate as a continuous record of the lathe turning rate was not obtained during onset and decay

Egmond Groen and Jongkees (1949) have suggested that a leak may occur in the cupula valve with impulse cessation of turning after exposure to angular velocities of 180 /sec. If the stopping time in their investigations were 0.5 sec, then the angular acceleration would be 360 /sec² On the other hand, McCabe (1964) has conducted intensity function studies which indicate that angular decelerations as large as 720 /sec² produce no evidence of vestibular abnormality

The weight of experimental evidence indicates that the static equilibrium reflexes are mediated by the maculae and that the semicircular canals are concerned primarily with compensatory reactions to angular acceleration (Gernadt 1959 Birukow 1959) Even if the angular accelerations applied in the present study produced damage to the semicircular canals, and McCabe s study indicates that this is not the case the abilities to perform the righting reflex and to swim should not be affected

Procedural Limitations

Apparatus

A number of procedural limitations must be considered when interpreting the results of these experiments. One of the major difficulties with

these investigations relates to limitations of the stimulating apparatus. Two types of problems were encountered with the SFAPS. First, the limitation of onset rate to 40 G/sec forced the neglect of a set of acceleration profiles which would be of considerable theoretical interest. Second, sometimes the animals' heads were displaced during exposure on the SFAPS. This means that for these animals, the direction of the acceleration vector relative to the maculae varied during the experimental run, which tends to confuse attempts to relate locus of damage to direction of acceleration.

Limitation of displacement with the vibration exciter restricted the profiles to which animals could be exposed in the vibration experiment. With the vibration exciter employed in the present experiment, the frequency had to be set at 6 Hz to obtain 1-G linear acceleration at the maximum displacement for the apparatus.

Behavioral observations

The behavioral observations used in these experiments do not provide a fine picture of vestibular damage. Performance of the righting reflex requires only a sufficiently intact receptor to signal the direction of acceleration due to gravity when the animal is placed on his back. However, this drawback is partially offset by the ease with which the righting reflex can be quantified.

Tests of swimming ability should provide a more complete picture of the extent of vestibular damage than the righting reflex. However, it is difficult to obtain a satisfactory quantification of swimming ability. Both swimming ability and the righting reflex are subject to compensation by other senses. We have observed animals which could not perform the righting reflex consistently when blindfolded achieve ten 4-point landings in ten drops when vision was restored.

Anatomical observations

Observation of damage with gross dissection is subject to artifact; no matter how carefully the dissection is performed, it is possible that the fine structures of the vestibular receptor may be disturbed. Therefore with this technique, it is only possible to note major alterations of the recep[illegible]

Possible histological artifacts include poor penetration of fixative, [illegible] decalcification and improper staining which can influence the appear[illegible] of the maculae and cristae in both control and experimental anim[illegible] preservation of the vestibular endorgans is frequently more diff[illegible] assure than that of the organ of Corti. In the same section it is [illegible] to observe a well preserved cochlear sense organ with fixati[illegible] present in the vestibular endorgans. A particular problem rel[illegible] age of the cupula which greatly increases the problem of [illegible] in the semicircular canals. Not only do sensory and sup[illegible] epithelium of cristae and macul e reveal vacuolization [illegible] cytoplasm and nucleus but the otoconial layer is less [illegible]

spheres of protoplasm are numerous in the interval between the epithelial surface and otoconia However when the bone is properly decalcified the otoconia prisms retain their shape, position and stain deep blue with hema toxylin There is no reason to question either their presence absence or distribution in experimental animals when compared with nonexperimental animals prepared by the same methods for which all factors have been adequately controlled

The incidence of otitis media was found to be relatively high particularly in postexposure animals allowed to survive for several weeks. Unless a purulent labyrinthitis was also present the sections were usually found to be suitable for reading The vestibular endorgans showed a minimum of changes that could be attributed to infection

CONCLUSIONS

The major conclusions which may be derived from this work are as follows

1 Behavioral loss, in terms of the righting reflex and swimming ability may be produced by exposure of guinea pigs to accelerations of 50 G for periods of approximately 60 sec. Accelerations below 50 G do not produce behavioral loss. Accelerations of 100 G for 30 sec result in consistent evidence of behavioral disturbance Exposure to 200 G over a triangular G-time history of 10–15 sec may result in behavioral loss. Acceleration exposures of more than 200 G for periods of 15–20 sec elicit consistent evidence of behavioral damage.

2. Light to moderate loss of otoconia from the maculae may be observed following exposure to 12–25 G for 105–330 sec. Moderate to severe otoconia displacement results from acceleration exposures of approximately 50 G for 1 min Accelerations of 100 G for 30 sec and of over 100 G for 15–20 sec produce severe otoconia loss from all maculae

3 Ability to perform the righting reflex and amount of otoconia loss are highly related, as is indicated by a product moment coefficient of correlation between these two variables of −0 69

4 Where righting reflex loss is produced by accelerations of less than 300 G, functional recovery usually occurs from 1 to 64 days after the exposure. Exposure to 400 G for 15–20 sec generally produces irreversible loss of the righting reflex.

5 No histological evidence for reformation of the otoconia was observed up to 70 days after exposure The hypothesis is suggested that the maculae may be functionally operative if the gelatinous layer remains intact or is replenished during the postexposure period

6 Behavioral and histological results indicated that acceleration perpendicular to the macular surface produces greater damage than acceleration parallel to the macular surface

7 Vibration at 1–2 G 6–8 Hz for 6 hours may produce transient behavioral disturbance Negligible to moderate otoconia displacement results from these vibration exposures

8 Exposure to noise at 140–154 dB SPL for 20 min does not produce disturbance of the righting reflex or swimming ability Anatomically utricular and/or saccular membrane collapse, as well as slight disturbance of otoconia may be observed following sound exposure at this level

9 Observations from this work support the hypothesis that "benign, paroxysmal, positional nystagmus" results from displacement of otoconia into the canal ampullae

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ZUSAMMENFASSUNG

Die Versuchstiere (Meerschweinchen) wurden entweder Linearbeschleunigungen, periodischen mechanischen Schwingungen oder hohen Schalldrucken ausgesetzt um sowohl die Funktionsstörungen als auch die pathologischen Veränderungen des Otolithenapparates zu untersuchen. Hierzu wurden zuerst Funktionsänderungen der Labyrinthreflexe als Folge der verschiedenen mechanischen Beanspruchungen untersucht danach wurden Sacculus und Utriculus sowohl durch direkte Einsichtnahme nach Öffnen der Knochenwand als auch histologisch an Hand von Zelluloid Serienschnitten untersucht Die Hauptergebnisse der Untersuchungen sind folgende

1 Die untere Linearbeschleunigungsgrenze bei der ein Verlust des Umdrehreflexes und eine Beeinträchtigung der Schwimmfähigkeit beobachtet wurde ist ungefähr 50 G für Dauer von 60 sec Beschleunigungen von 100 G für 30 sec resultierten in dem Abreissen eines beträchtlichen Teiles der Otolithenkristalle in allen Maculae.

2. Obwohl höhere Reizintensitäten nötig waren um nachweisbare Funktionsänderungen zu beobachten als um pathologische Zerstörungen zu erzielen ergab sich eine hohe Korrelation zwischen Störungen des Umdrehreflexes und der Otolithenschädigung.

3. Nach Belastungen bis zu 300 G für 15 sec normalisierten sich Schwimmfähigkeit und Umdrehreflex über einen Zeitraum von 1 bis 69 Tagen. Nach Belastungen über 400 G für 15 bis 20 sec ist der Verlust des Umdrehreflexes irreversibel und die Schwimmfähigkeit bleibt schwer beeinträchtigt. Der hist logische Befund zeigte k ine Anzeichen von Neubildung von Otolithenkristallen bis zu 69 Tagen nach der Belastung Jedoch wird die Möglichkeit erörtert, dass sich die gallertige Schicht der auf den Sinneshaarzellen gelagerten Otolithenmembran neubildet.

4. Wenn der Beschleunigungsvektor rechtwinklig zur Macula Oberfläche einwirkt, führt er zu schwereren Schädigungen als wenn er senkrecht zur Macula Oberfläche orientiert ist.

5 Schwingungsbelastungen von 1–2 G Spitzenwert und 6–8 Hertz für 6 Stunden können zu vorübergehenden Beeinträchtigungen der Verhaltensleistungen und zu geringen Verlagerungen von Otolithenkristallen führen

6. N ch Beschallung mit Breitbandlärm von 154 Dezibel Schallpegel für 20 min liessen sich keine funktionellen Störungen n chw isen nur geringfügige Verlagerungen von Otolithenkri tallen wurd n beobachtet.

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SUPPLEMENTUM 240

EPIDERMAL MIGRATION IN THE EAR. THE LOCATION AND CHARACTERISTICS OF THE GENERATION CENTER REVEALED BY UTILIZING A RADIOACTIVE DESOXYRIBOSE NUCLEIC ACID PRECURSOR

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SUPPLEMENTUM 240

EPIDERMAL MIGRATION IN THE EAR THE LOCATION AND CHARACTERISTICS OF THE GENERATION CENTER REVEALED BY UTILIZING A RADIOACTIVE DESOXYRIBOSE NUCLEIC ACID PRECURSOR

W B LITTON

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INTRODUCTION

What is probably the single most important characteristic of the external ear epidermis, its proliferation and migration, is never alluded to in modern monographs or textbooks dealing with the ear This property has been sporadically investigated for at least three quarters of a century but few clinicians have been aware of its everyday implications. This report attempts to elucidate the exact physiologic mechanisms governing epidermal migration, to relate this cytokinesis to that of other organ systems, and to point out the importance of this complex and fascinating bit of otology to the clinician. Lastly new paths of research will be envisioned efficient assimilation of all this knowledge may assist in medical and surgical treatment of the ear

The most obvious manifestation of epidermal migration the movement outward over the tympanic membrane and external canal of foreign materials, has probably been noted since otoscopy began The first references in American medical literature are found almost 100 years ago C. J Blake (1882) noted the migration of paper patches placed over perforations in the tympanic membrane He noted that the observation of foreign body migration was not new but proceeded to clarify his clinical observations by experimentation The results of his experiments which consisted of the attachment of bits of glazed paper to the tympanic membrane and periodic inspection for migration patterns, were new he felt and correspond with those of more recent times. The patches migrated from the umbo outward but seemed to prefer a swirling pattern to the superior and posterior canal wall whence they progressed to the cerumen bearing canal

Blake s statement admitting the fact of previous wide spread knowledge of the migration of aural foreign bodies is confirmed by Buck (1880) in his textbook who mentions this epidermal property as assisting in the prevention of cerumen impactions Later textbook authors who included this explanation of normal canal physiology include Burnett (1881) and Bezold *et al* (1908)

Blake (1900) apparently maintained his interest in this bit of physiology and in 1900 extended the clinical implications of the lack of normal epi dermal mobility

> In the chronic forms of epidermal disturbance which are a common sequence of the acute attacks more commonly observed in adults, the epidermal layer seems to have lost its normal progression or to have had it so much impaired as to result in the accumulation of super imposed epidermal layers in situ the result being, in some instances, a mass of epidermis, in more or less well defined strata nearly or wholly filling the inner half of the auditory canal the keratosis obturans of Wreden

Link (1952) studied the blood and lymph vessel distribution in the tympanic membrane and external canal in association with the pattern of movement of ink dots placed on the tympanic membrane He noted the radial distribution of vessels as well as the radial progression of dots out ward and implied a cause and effect relationship without clarifying the coincidence

Stinson (1936) observed ink dot migration too but it is unclear as to the exact pattern that emerged from his observations He stated the migra tion was from anterior to posterior over the tympanic membrane and

canal and noted no canal to drum migration He related his observations to the healing of membrane perforations stating that the anteroposterior migration accounted for the poorer healing of anterior central perforations.

Epidermal migration and its relationship to cholesteatoma has aroused much speculation Magnoni (1938) repeated Blake s experiments on human foreign body migration and concluded that cholesteatoma of the middle ear could not originate from the canal because no migration was ever noted of epidermis in a canal to drum head direction. Nevertheless Simmons (1961) employed the clinical test of ink dot migration into the middle ear to detect cholesteatoma in central perforations.

More recently there has been a renaissance of interest in canal and tympanic membrane lining characteristics This has attended the development of aural microsurgery tympanoplastic surgeons have developed manipulative techniques requiring this knowledge for successful completion of reconstructive efforts.

Litton (1963) illustrated the normal pattern of epithelial migration and noted its rate to be 0 05 mm per day The success of canal skin in myringoplasty over skin from other areas was attributed not only to its lack of accessory skin structures, but to its retention of epithelial mobility resulting in self cleansing He noted low rates of migration in persons with chronic external otitis, much as Blake did 81 years previously

Alberti (1964) made careful clinical investigation of ink dot migration patterns and rates and has related this phenomenon to clinical practice He noted an average rate of migration of 0.07 mm per day and also noted the umbo to be the center of migration. The importance of this property to aurists was in its accounting for the self cleansing property of the ear canal and for the success of canal skin myringoplasty This author felt that chronic external otitis resulted not from a defect of epithelial migration but from an abnormal proliferation of epithelium in a vertical direction which overwhelmed the lateral migratory rate and resulted in desquamation This author doubted any relationship of epidermal migration to middle ear cholesteatoma because such migration would tend to prevent, rather than cause such an epidermal cyst

In summary the effects of epidermal migration over the tympanic membrane and external auditory canal in expelling foreign bodies from the external canal have been noted since systematic otoscopy began The pattern and rate of migration are known this rate is rapid (about 0 05 mm/day in the human) and the pattern in normals quite constant (outward in humans from the umbo) Clinicians have been interested in the following implications of these basic observations

1 This property keeps the canal open and free of keratin or sebaceous or cerumenous debris. Keratin is not sloughed into the lumen of the canal but is carried outward by the lateral rather than vertical movement of this specialized epithelium

2 Epidermal migration is essential for tympanic membrane and canal healing The basic normal migratory properties of this special epidermis are undoubtedly altered by trauma. Nevertheless the resumption of a pre-trauma pattern by homogenous tissue is necessary for the continued health of the ear Epidermis without migratory properties cannot be substituted in reconstructive operations.

3 Persons with chronic desquamative external otitis have been observed to have a defect in migratory rate. The end point of this failure may be keratosis obturans.

4 Some epidermal cysts of the middle ear may arise from tympanic membrane or external canal skin an understanding of the exact mechanism of production of such cholesteatomas, especially when the drumhead is intact awaits more knowledge of normal external ear physiology

Since the underlying physiologic principle explaining "epidermal migration" has not been uncovered and a better understanding of its basic mechanism would be of value an experiment was designed to elucidate it in the guinea pig tympanic membrane and external canal

HYPOTHESES FOR THE MIGRATION MECHANISM

A number of mechanisms might be postulated to account for the movement over these structures of substances placed on the tympanic membrane and canal

1 *Lymph flow hypothesis*

Link (1932) noted that the ink dots he placed on the human tympanic membrane moved outward in the same direction as the vascular channels He therefore proposed that the movement of the lymph in some way propelled the epithelium outward The exact mechanism involved was not explained

2 *Vibration hypothesis*

Many individuals consulted by the author favored a vibratory mechanism for the expelling of foreign materials placed on the eardrum Because the membrane vibrates with maximal amplitude near its center with decrements of movement more peripherally (Bekesy 1941) when struck by low frequency sound waves, material might be carried towards its periphery by such vibration. However this explanation does not serve for the osseus canal which is rigid and yet transfers superfically placed foreign bodies. Stinson s (1936) observations of the migration of an embedded twig and the daily contemporary observation of the rapid expelling of large plastic tubes placed *through* the tympanic membrane also tend to discredit this as the operative mechanism.

3 *Whole organ growth hypothesis*

Perhaps the whole organ is growing maximally at some point and in its continual renewing pushes the lining outward All three layers would participate in such movement and an explanation is offered for some observations of whole thickness membrane perforations migrating off the drum and out the canal This theory is made unlikely by observations recorded under Results.

4 *Ameboid movement hypothesis*

One could postulate active ameboid movement of superficial squamous cells over more deeply placed cells. This would conflict with behavior of skin elsewhere which migrates upward by a differential rate of growth with the basal cells reproducing at a maximal rate and pushing the cells upward Electron anatomical studies of body skin elsewhere dem nstrates

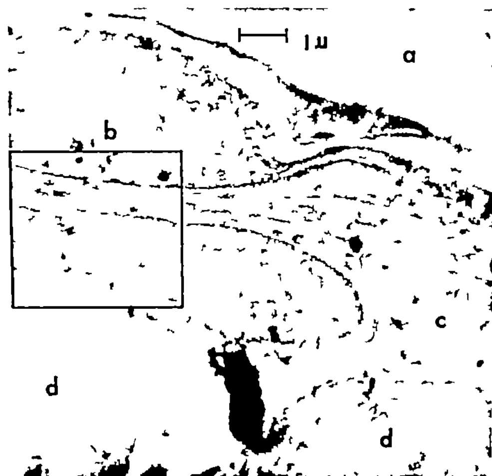


Fig 1 Thi l w power lectron micrograph l cross section through the guine pig tympa l membrane: a External anal; b keratinized cell f pidermal layer; basal cell epidermal l ver d cros section f connecti tis fibril f middle tympanic m mbrane l er The recta gul rea t the l ft l magnified l the foll wing micrograph.

myriad desmosomal interspaces (the "intercellular bridges" of light microscopy) locking the cells together and preventing lateral movement (Odlund, 1958) The guinea pig tympanic membrane squamous epithelium was studied by electron microscopy and many desmosomal connections demon strated (Figs. 1 and 2)

5 Generation center-differential growth hypothesis

Movement between the central connective tissue layer and the epidermal layer is postulated The mechanism for such movement may be a dif ferential rate of epidermal growth with the center of migration representing an area of most intense mitotic activity This activity pushes the epithelial layer centripetally from the generation center This would explain the retention of ink dots on the surface without slough into the canal lumen

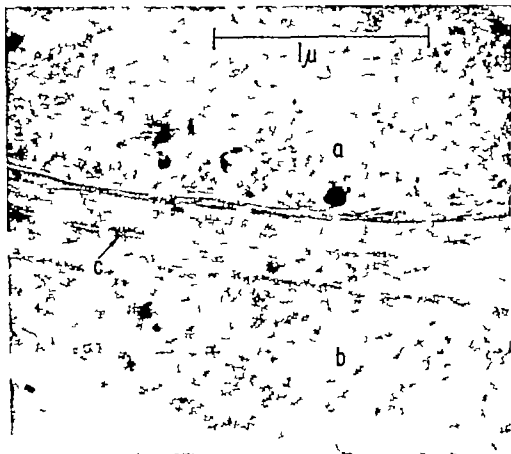


Fig 2 High power i estig tion of epidermal layer indicated in Fig 1 guinea pig TM pidermis, reveals multit de of cell m mbrane plates binding cell togethe bov the connective tissue level a, Cornified cell; b cell nucleus of basal layer pidermis c, t no-fibrils pproachi g cell membrane plates ("intercell la bridges of ki)

there is only enough vertical proliferation of squamous cells to maintain an optimal epidermal thickness. This optimum thickness is slight over the tympanic membrane (see Fig 1) in order to allow maximal vibrating sensitivity It is progressively thicker over the canal to provide protection of the underlying bone and cartilage.

EXPERIMENTAL METHOD

India ink dots were placed at various locations over the adult guinea pig tympanic membrane and external canal. Because of the very narrow isthmus of the guinea pig's canal, dots could not be placed in all peripheral locations. Nevertheless, after 44 ink dot applications to 13 guinea pigs and observations at daily intervals for periods up to 10 days the pattern of motion seen in Fig. 3 was perceived. The guinea pig center of migration appeared to lie posteroinferiorly, inferiorly, and slightly anterior *in the canal* close to the tympanic membrane. Particles were propelled outward from this center. This contrasts with the human pattern of radial migration from the umbo.

Accurate measurements of particle velocity were difficult, but by comparing dot migration distance with malleus handle length an average rate of 0.07 mm for 24 hours was established over the tympanic membrane. This estimate is based on 30 measurements in 10 different guinea pig ears. The range was from 0.10 mm to 0.08 mm per 24 hours. It should be noted that this rate is approximately ten times that observed in the human. Rates of migration over the canal were impossible to measure because of inaccessibility to view.

As interesting as the tracing of foreign body migration over the tympanic membrane and canal has been over the past 100 years, it indicates keratinized layer movement at the most. *If the secret of the physiology underlying these fascinating wanderings of twigs, papers, inks etc. is to be known, individual cells must be labelled and followed. Furthermore, some method of identifying proliferating epidermis as opposed to static or resting skin must be devised.* In 1957 Taylor *et al.* (1957) first employed thymidine, a desoxyribose nucleic acid building block which could be tagged by a radioactive isotope of hydrogen, to study the generative cell cycle. This technique has provided valuable basic data about cellular reproduction in all organ systems. Cell populations have been divided into three categories (Messier *et al.*, 1960; LeBlond *et al.*, 1959; LeBlond *et al.*, 1950): (1) *Static cell populations where no cell division is detected.* The cerebrum is an example. (2) *Expanding* cell populations are marked by cellular division without loss of constituent cells. The eye's lens is an outstanding example (Harding *et al.*, 1960). (3) *Renewing* cell groups are continually produced and lost to the outside. Cell division is most abundant in this category (Messier *et al.*, 1960; Schultz *et al.*, 1960; MacDonald *et al.*, 1950). Examples are cells of intestinal mucosa, of the bone marrow, of the testes and of the skin.

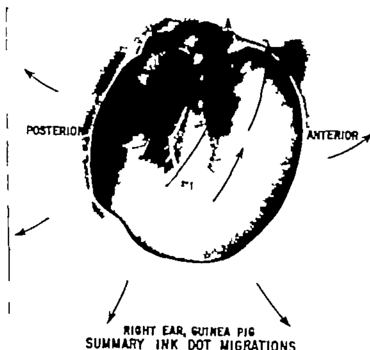


Fig. 3. This is summary f ink dot migrati pattern on guinea pig drum head If the generati n center-growth differential hypothesis explains these findi gs, the growth center must be about the m rgins of the tympanic membrane anteriorly i feriorly nd posteri rly

Thymidine is a complex protein molecule which is incorporated into desoxyribose nucleic acid (DNA) and into no other cellular constituent DNA is found only in the cell nucleus in contrast to the other nucleo-protein, ribose nucleic acid (RNA) which may be found in cytoplasm (Brown 1956 Swift 1950 Reichard *et al* 1951) Therefore, a cell assimilat ing thymidine is preparing to duplicate its nucleus (Amano *et al.*, 1959 Messier 1959)

Cell generative cycles may be divided into phases (Quastler *et al* 1959 Sherman *et al* 1959 Baserga, 1965) Fig 4 illustrates schematically how one may think about such a life cycle with the area of the circle correspond ing to the time consumed in each phase *S* phase represents the time spent in synthesis of DNA It is here that thymidine as well as other nucleo-protein building blocks, is taken into the cell. *G2* is a premitotic phase assimilated nuclear materials are being put into a condition for the *M* or mitosis phase *G1* represents differentiation toward "adulthood and the specific function of the tissue type

Some cells never differentiate, but continue to function as "parents" or reproducers of the lineage These cells may be called the generative cell fraction in contrast to the maturing cell fraction This concept is schema tized in Fig 5 Our hypothesis concerning epidermal migrati n over the

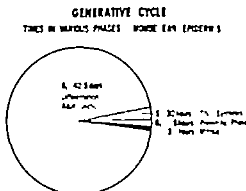


Fig 4 Times in arlou ph ses of epide m l generati es l re proporti l t ircle rea l dicated for each

guinea pig tympanic meml rane and external canal would concentrate a generative cell fraction deep in the ear canal or over the drum head

Thymidine may be prepared so that it carries a tritium label (Bertalanffy 1964) Tritium is the H3 is tope of hydrogen and is radioactive (Evans,

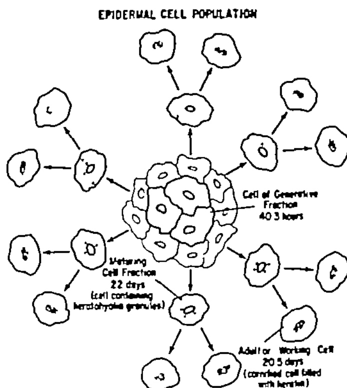


Fig 5 Thi schem of the division of labo mongst cell t pe population m st obviously be i fluenced by ma y variables. Physical disruption m st allow some mat ri g cells t ret rn t reproductive capacities. Neopla l i xtreme example of alteration

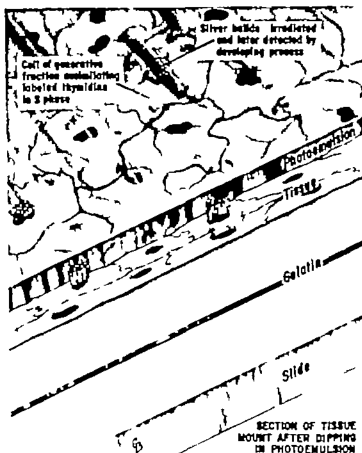


Fig 6. The il er granules are deposited ov radioacth uclei. The tiss sections have been implified; n inner l gle layer f noncornifyi g cell is next t the gel tin nd the co nective tissue (*middl*) layer f TM l interposed between these two epithelial layers i the tympanic membran specimens act lly examined.

1966) Radioactivity may be divided into alpha beta, and gamma varieties. Alpha radiation is particulate and consists of helium nuclei (2 protons, 2 neutrons) The particles velocity is slow with very low penetrance Beta radiation consists of electrons and is the type given off by tritium Its velocity and penetration potential is greater than alpha radiation but much less than gamma which is not particulate but is in the electromagnetic wave spectrum Ordinary x rays are electromagnetic waves with longer wave lengths than those of the gamma spectrum (Shilling 1960 Blahd 1965)

Tritiated thymidine as well as other DNA precursors, will be taken into cells preparing to divide The cells whose nuclei are later discovered to contain radioactivity (by a method to be explained) were in the S phase of the cell generative cycle at the time of tritiated thymidine administra tion and subsequent availability to the cell population under study Studies

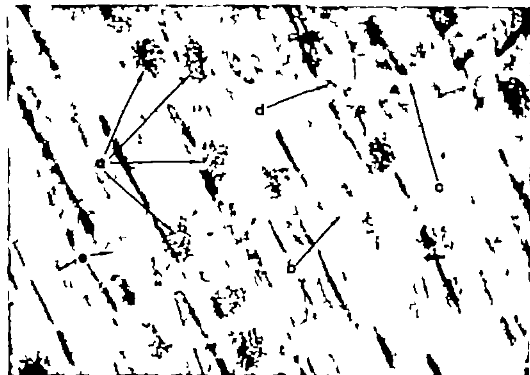


Fig 7 Thi l a high dry (433) photograph f 1tl hoe tymp l membrane specimen after completion of preparation Nucl labels (a) l thi tympa ic membrane cor respond closely t the fl tt ed epidermal nuclei. Larger lighter gra ules re keratohy line (b) Long a rrow ucl i (c) re l co nectl e ti sue l yer Endothelial cell (d) are seen l wall f curving blood vessel

have shown prompt excretion of this material in 20 to 60 minutes a single dose is available to S phase cells for only a short time (Baserga, 1964) *A pulse label of such nuclei results from* intravenous, intraperitoneal or subcutaneous administration of tritiated thymidine This label is bound tightly in the nucleus and is not washed out by any solvents It is diluted only by division of nuclear substance during succeeding mitoses.

Beta radiation is given off for a long time (tritium half life is 12.26 years) by cells incorporating radioactive thymidine into their nucleus during the 60 minutes following its administration (Bazley *et al* 1965) The presence of beta radiation can be detected by the changes the irradiation produces in photographic emulsion Fig 6 is schematic and summarizes the position of tissue photographic emulsion and silver particles produced in the developed and fixed emulsion layer Fig. 7 is an actual high dry light micrograph of a flattened section of guinea pig drum head showing nuclear labels The observer is looking from above through the very transparent, lightly counterstained membrane The epidermal layer is uppermost beneath the photoemulsion

The method of dispersion of radioactive isotope as successive cellular divisions occur is summarized in Fig 8 The original aliquot of radioactivity

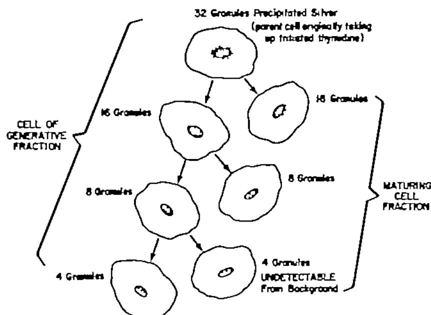


Fig. 8. Each division of cell originally taking up radioacti thymidi will result in hal i g of its radioactivity Those first order "sons not redividing will retain thei labels (for example, the t p two cells in maturing cell fracti n)

appearing in the first generation cell (the cell in S phase at time of ad ministration of tritiated thymidine) will be halved with each subsequent division It is obvious that at some point the radioactivity will become undetectable from the emulsion background as the original mother cells divide and as their descendents redivide

While detectable nuclear labels remain an idea of the place in the line of descent should be determinable by the amount of radioactivity in the nucleus compared to the cells with the maximum uptake A quantitation of radioactivity is achieved by counting the number of precipitated silver par ticles above each labelled nucleus.

Guinea pigs were administered 1 microcurie of tritiated thymidine per gram of body weight subcutaneously One half the calculated dose was de-posited behind each external ear Three animals, resulting in six tympanic membrane specimens and six canal skin specimens were enlisted in each time category except one day specimens where two animals were sufficient The total number of guinea pigs was therefore twenty with 80 separate specimens. The animals were sacrificed at intervals of four hours, one day two days, four days, six days, nine days, and twelve days These times, however were irregular and are expressed on the final diagrams in hours post injection

The tympanic membranes and canal epithelium approximating the drum



Fig 9 Photomontag f the whol tympa ic membrane mount sed t precisely place nuclea label on the pecimens. The actual iz f the montage is 36 27 inches. The "map ffect f the branchi g scul pattern I cl ly isibl Thi di ides the mont g I I "con ties each with quantifiabl number of l bel according t n mber d lineage

were dissected out and placed epidermal layer upmost on gelatinized slides after fixation in neutral formalin They were dried and then covered with photographic emulsion by the dip technique They were incubated in the *dark for two to four weeks and then developed, fixed and stained with* hematoxylin and eosin Fig 9 is a whole mounted tympanic membrane The canal skin specimens were not counterstained so that the labelled nuclei would be better shown Fig 10 is a whole mount of canal skin originally adjacent to tympanic membrane

The specimens were photographed in multiple fields employing enlargements of about 100 times. The photographs were fitted together to enable study of the whole organ and taped in position on fiber board backing Each tympanic membrane had a distinctive "map" occasioned by the capil



Fig. 10. The canal skin specimens incl de th four millimeters of epidermis nd dermi closest t the TM. The upper portion could not be excised in continuity in most cases. Labels can be seen; the specimens re unstained.

lary network (Fig 9) Each county" enclosed in a capillary boundary was scanned under high dry magnification and each labelled nucleus placed on the map on a sheet of transparent plastic placed over the assembled micrographs Canal skin nuclear labels could be detected on the photographs of the whole mount and their positions were marked directly on the transparency

In the specimen summaries to follow each dot represents a labelled cell. "Labelled cell" refers to a nucleus whose deposit of silver granules was definitely separable from the background No attempt was made to quantitate the number of granules per cell, as this would occasion several years of work. Nevertheless, impressions of relative position in cell lineage always agreed on check scans with the direction of migration as postulated by the ink dot movement and the deductions of position of generation center and subsequent growth patterns This is, heavier labels occurred at the

advancing edge of migration the generative fraction of cells gradually lost their label

In summary the experimental method was as follows

1 *Pattern* and *rate* of *keratin layer movement* in the guinea pig was identified This was done by conventional india ink dot stippling and observation of ink dot movement at intervals post application of dots

2. *Individual live cell labelling* was accomplished with a radioactive DNA precursor tritiated (hydrogen isotope) thymidine To be precise the nucleus of cells about to divide in a very short time segment (1 hour) were labelled by incorporation of radioactive thymidine into the nucleus. Animals were sacrificed at various intervals after cell labelling to allow for these cells migration and the later deduction of migration steps

3 *Detection of labels* was carried out by autoradiographic techniques at stated intervals after the short labelling period and the position of labels noted on the whole specimens.

4 *Deduction of growth pattern* was allowed by the above maneuvers and was summarized by appropriate figures. The use of tritiated thymidine fulfilled both our demands as underlined in the statement on page 12 individual live cells could be detected and movements traced and the generative fraction of cells could be detected This pattern can then be compared with keratin layer movement the generation center-growth differential hypothesis may thus be directly tested It must be emphasized that we have chosen only a small segment of the cell population for our observations. The growth and renewing of the epidermal cell population over the tympanic membrane and external canal is a continual flowing process, never ending and probably subject to many external influences and to individual and species differences.

RESULTS

India ink tattoos were placed in a guinea pig tympanic membrane Ink was deposited on the surface as well as through the connective tissue substance A more superficial bit of ink was seen to migrate away from the more deeply placed foreign material, which remained embedded for observation periods of one month. In the experiment to be described below very few elongate connective tissue cells were seen to take up thymidine This indicates that multiplication of such cells is rare in the intact drum head The same observation was made for middle ear mucosa stripped from the inside of the tympanic membrane and prepared separately with photoemulsion.

The specimens of tympanic membrane four hours post-injection (Figs. 11 12 and 13) show very few labels. Those seen are at the periphery anterosuperiorly and posterosuperiorly However immediate (4 hour) canal skin specimens show heavy uptake near the tympanic annulus posteriorly an teriorly and inferiorly with few labels high in the canal The canal skin from 11–1 o clock could usually not be retained with the other skin and was placed separately on the slide Therefore, many of the summarized specimens, including Fig 14 do not include this bit of epithelium These results are summarized in Fig 15 Each summary figure is necessarily a conceptualization. Greater and lesser uptake densities are indicated but are never sharply demarcated in actuality Uptake densities in the summary figures are not meant to be comparable between interval summaries. These summaries are merely intended to mate the canal and tympanic membrane and allow correlation of nuclear labels on the two specimens.

By 98 hours a heavy band of labels appears at the tympanic membrane margin inferiorly and posteriorly There are scattered lighter labels along all margins and centrally This indicates second and third order division probably from the original generative fraction located in the posterosuperior and anterosuperior quadrants of the four hour specimens. Canal skin speci mens show labelling throughout the width of the resected specimen cel lular proliferation is driving the epithelium outward in the canal and upward over the drum head (Fig 17) Fig 18 summarizes.

The 144 hours specimens are revealing Fig 19 being representative Heaviest labelling over the tympanic membrane now occurs inferiorly and posteriorly The weak labels elsewhere have been lost due to dilution of radioactivity by cellular division as explained above. The cells whose labels we can detect at this time have matured and not redivided and their progres sion over tympanic membrane and canal will help clarify the growth pat

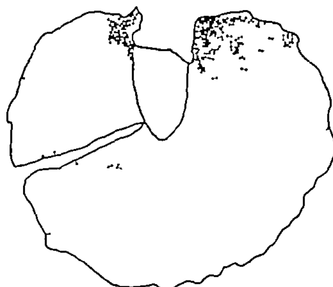


Fig 11 GP 101 tympa ic membrane w take from guinea pig sacrificed fou hours fter admi i trati f triti ted th midi e Each dot i thi figure nd those t f llow represe t nuclea label



Fig 12. GP 5L TM, fou hours



Posterior Anterior

Fig 13. GP 8R TM, four hours

CANAL EDGE

TM EDGE

Anterior Posterior

Fig 14. GP 10R canal skin pecimen, f hours.

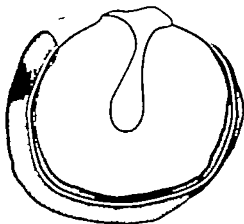


Fig 15. Summary of f ur hour pecimen fter re pproximati n t match TM in livi g nimal. The da ker tippling represents heaviest concentrati n f nucle whil the lighter tippled area ha fewer labels

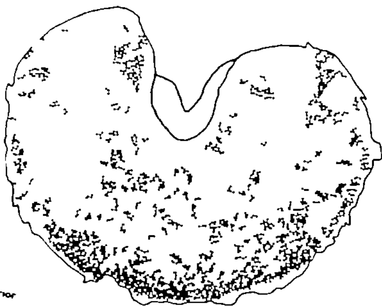
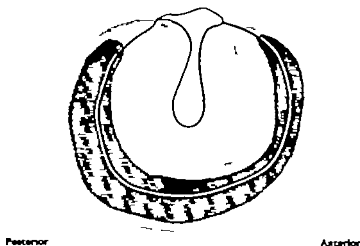


Fig. 16. OP 16L TM 98 hours after i jection



Posterior Anterior

Fig 17 GP 16L canal skin, 98 hours.



Posterior Anterior

Fig. 18 S mmary of label density t 98 hours. Hea lest nuclea label density is represented by darker shading

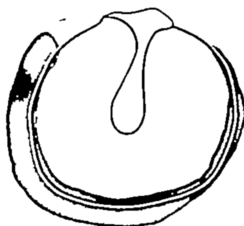


Fig. 15 Summary of fou hou specimens after re ppro imati n t match TM and canal in living animal Th darker stippling represent heaviest concentration of nuclear labels whil the lighter tippled area ha fewer l bel

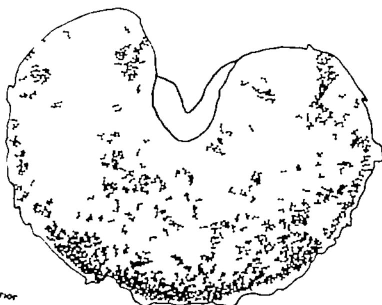


Fig. 16 GP 16L TM 95 hours fter injection.

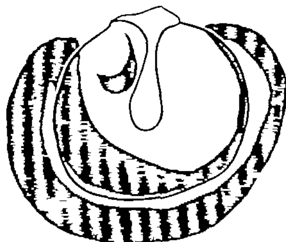


Fig 21 S mmary of 144 hours pecimens. Darker shading represent highest th midin uptake The lighter reas ha fewer radioacti uclei.

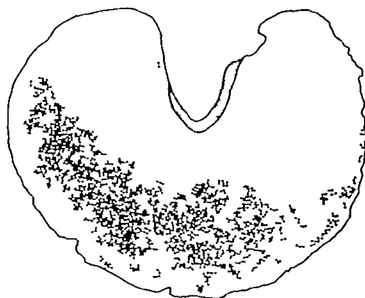


Fig 22. GP 17R TM 221 hours fter injection tritiated thymidin



Fig. 23. GP 18R TM, 221 hours.

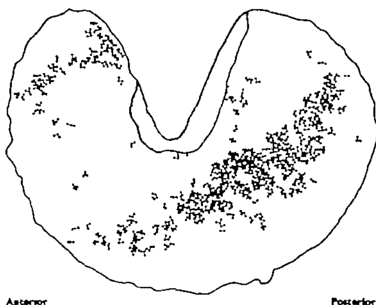


Fig. 24. GP 18L TM, 221 hours.

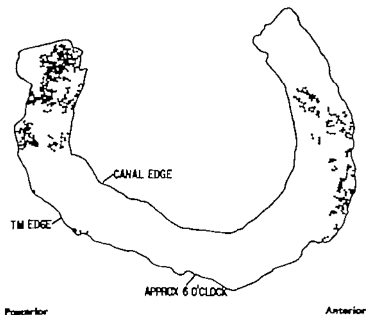


Fig 25 GP 17L canal kin, 221 hours

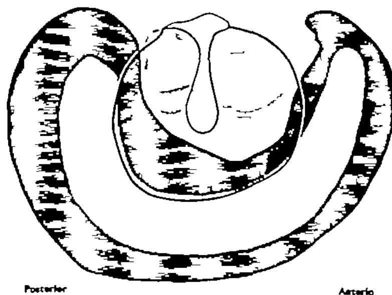


Fig. 26 S mmary of 221 hour specimens. Shading is roughly proportional t l belled nucleus density

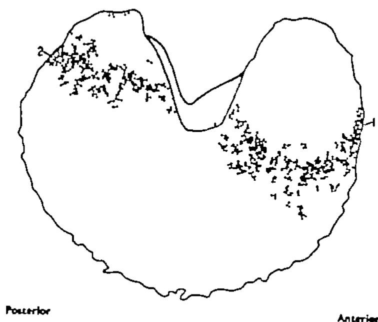


Fig. 27 GP 19R TM 238 hours.

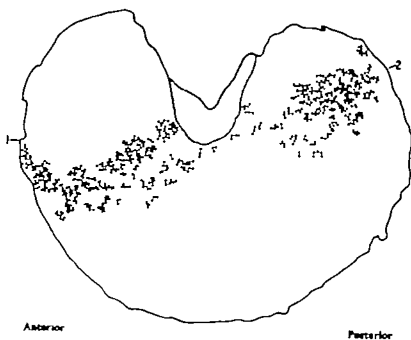


Fig. 28 GP 19L TM, 238 hours.

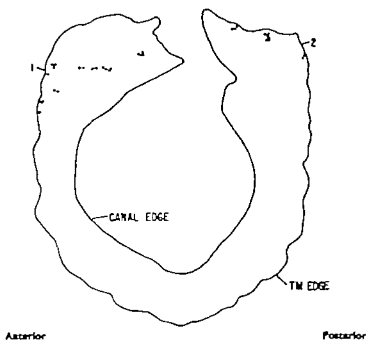


Fig 29 GP 19R canal skin, 288 hours.

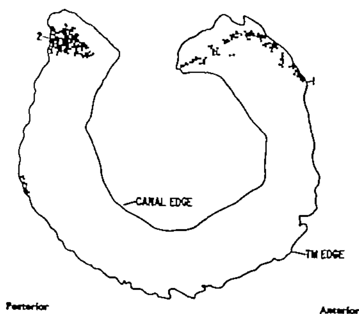


Fig 30 GP 19L canal skin, 283 hours.

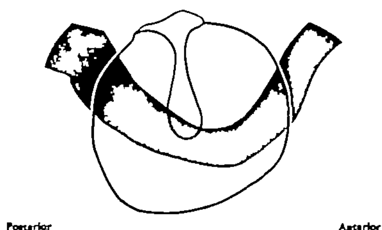


Fig. 31 Summary of 238 hour specimens.

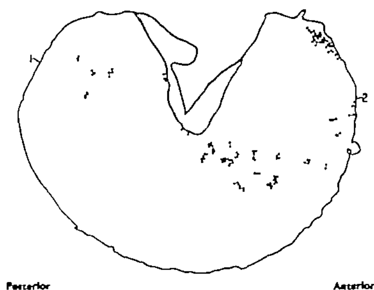


Fig. 32. GP 20R TM 318 hours

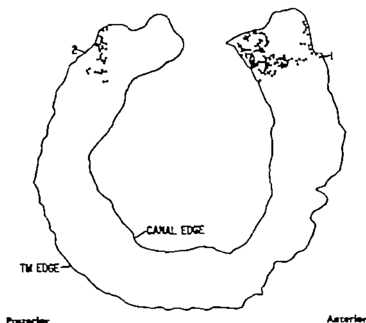


Fig 23. GP 20R canal skin, 218 hours

tern. The heavy uptake anteriosuperiorly must represent an amalgama tion by differential growth rate of labels originally seen in this same loca tion in the four hour specimen The labels anteriorly and posteriorly may be a band across the malleus labels cannot be separated from bone desity here The canal skin specimens (Fig. 20) show fairly uniform distribution of labels throughout, but become relatively more dense peripheral to the generative center (toward the canal edge) Fig 21 interprets these changes.

Figs 22, 23 and 24 are representative drum heads removed at 221 hours after injection. The band of labelled nuclei has progressed across the membrane This band also appears in the canal skin (Fig 25) The labels

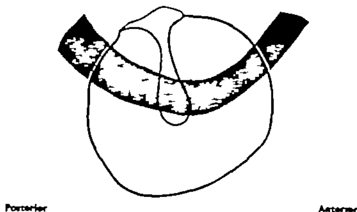


Fig. 24 Summary of 218 hour pecimen

indicated as a band far out in the canal are postulated, no other pattern could be reasonably deduced

Tympanic membrane specimens removed at 288 hours (Figs 27 and 28) demonstrate a band of radioactive nuclei higher over the tympanic membrane and their fellows are likewise seen as a continuation of this band over the canal skin (Figs. 29 and 30) These labels have travelled about 6 millimeters in 288 hours for an average rate of 0.50 mm per 24 hours This rate and direction of migration corresponds closely to those of the ink dots. An interpretation is seen in Fig. 31 The advancing wave far out in the canal is not shown

Figs 32 and 33 illustrate the fact that labels are still detectable high over the tympanic membrane and canal skin at 318 hours Fig 34 is the final summary

INTERPRETATION OF RESULTS

The experimental results support the concept that skin moves over the tympanic membrane and canal More significantly it supports the hypothesis that a differential growth rate of skin from the generation center centripetally is the mechanism of this skin movement The generation center or area of most intense mitotic activity takes place in canal epidermis just distal to the annulus in the guinea pig By analogy this center in the human is at the umbo

The mitotic activity at the generation center is intense with mitoses occurring elsewhere only in sufficient numbers to attain a suitable epidermal covering both in thickness and area. The radioactivity index, a count of the number of labelled nuclei compared to the total number of nuclei in the area, was 19% in both four day and six day specimens over the lower tympanic membrane This placed the mitotic activity of skin in the generative area second only to that observed in duodenal epithelium It is about 2 ½ times greater than the activity in the basal layer of the epidermis elsewhere The proliferation rate in this quiet unabraded area exceeds that of tongue epithelium (Odlund, 1958 LeBlond *et al* 1956) In citing these figures we have compared guinea pigs with mice and rats.

This organ system moves the epidermis over the underlying connective tissue in a manner analogous to the specialized epidermal structure of the fingernail It is obvious that some such mechanism must obtain in this cul de sac of skin if canal plugging by keratinaceous debris is to be avoided. The thinness and hence pliability of the tympanic membrane is thus assured vibratory sensitiveness is maintained A complete canal lumen free of debris and cleaning itself of any foreign particles escaping the vigilance of cerumen gland products and meatal hair is guaranteed. A remarkable physiologic mechanism of extreme fineness takes place daily before the otologists eye A prosaic bit of anatomy to the daily observer takes on an unanticipated complexity and, reveals Nature s cunning matching of structure and function.

A slowing or cessation of this specialized function may be reflected in two disorders. Ink dot migration has been observed to be very slow in persons with chronic external otitis (Litton, 1963) Blake (1909) seemed to agree as the result of observations made many years previous to the above and stated that keratosis obturans was the end point of such a disturbance with complete cessation of lateral epidermal migration and continuing vertical proliferation Other opinion of recent times on keratosis obturans holds it to be related to bronchiectasis and reflex hyperemia of the ear canal (Mor

rison 1956) Though the author has never seen a case for testing the explanation of migration failure seems more plausible

The proliferation of epidermis over the tympanic membrane and canal is of great interest to the aural surgeon He daily observes the results of such proliferation in the healing of wounds produced by physical force or infection He depends on this mechanism (as it is modified by tissue reparative processes) for the repair of tissues he has rearranged particularly in myringoplasty and tympanoplasty he has relearned those canal skin properties which make it most desirable for repairing canal or tympanic membrane defects (House *et al* 1961 Wastenson 1961 Guilford *et al* 1965) Freedom from accessory skin structure cholesteatoma and retention of migratory properties are important in achieving the best results in reconstructive ear surgery Further efforts expended on the study of the effects of variables on this rate of proliferation (e g age steroid preparations applied topically irradiation infection etc) might lead to even higher "take" rates in tympanoplasty grafting An objective basis for these studies which can be carried on outside the operating room is offered in this description of normal events.

One cannot help conjecturing on the relationship of some types of cholesteatoma to abnormalities of rate migratory direction or site of epidermal proliferation *Why do some tympanic membrane retraction pockets lead* to cholesteatomas in the mesotympanum or epitympanum while others do not? Many otologists have commented on the possibility of an external ear or tympanic membrane origin of some cholesteatomas (Austin *et al*., 1964 Harpman 1953 McGuckin, 1960) Though the normal epidermal migration rates and pattern would tend to prevent cholesteatoma formation abnormalities of this mechanism, (for example previously induced by infection) could account for some of these puzzling clinical observations Again it is hoped that this description of experimental method might be extended to the *patho*physiology of otic epidermal proliferation and migration

CONCLUSION AND SUMMARY

Epidermal migration over the tympanic membrane and external auditory canal occurs by differential growth rates deep in the canal of the guinea pig as determined by radioisotope labelling and identification of the fate of cell sequences. This is a very rapidly proliferating tissue whose proliferation, however is orderly and results in the maintenance of a thin pliable lining over the vibrating tympanic membrane and a full unobstructed lumen to the external auditory canal There is every reason to expect a similar mechanism to occur in the human the center of proliferation must be at or near the umbo in man The migration rate in man is about one-tenth of that observed in the guinea pig.

This experiment explains events in a normal animal Later studies should concentrate on factors influencing this mechanism such as irradiation drugs, age trauma infection, etc. A relationship between this normal mechanism and the production of cholesteatoma in the middle ear or attic may exist

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PERSTIMULATORY SUPRATHRESHOLD ADAPTATION FOR PURE TONES

I *Basic Studies on Normal Hearing Persons*

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SUPPLEMENTUM 241

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PERSTIMULATORY SUPRATHRESHOLD ADAPTATION FOR PURE TONES

I

BASIC STUDIES ON NORMAL-HEARING PERSONS

JUHANI KÄRJÄ

OULU 1968

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Kirjapaino Osakeyhtiö Kaleva
Oulu 1968

CONTENTS

I INTRODUCTION

Decreased response to sustained stimuli is a common feature of all receptors of the sensory system It can be objectively demonstrated, for instance by electrophysiological methods, as a decline in the rate of discharged impulses per time unit. The phenomenon is known as adaptation and, according to Ranke's theory its essential object is the production of a purposeful balance between stimulus and response to stimulation (Ranke 1955 Keidel 1961) Adaptation however is not merely a peripheral event, it seems to occur in the central nervous system too (Keidel 1958 Keidel et al. 1960)

Adaptation in the human ear is easily demonstrable as a shift in threshold of hearing, or as a decline in loudness of suprathreshold stimuli or it may appear in the form of changes in sound quality sound localization and in masked pure tone threshold. In animal experiments the phenomenon shows itself as a diminution in the auditory nerve action potentials and a reduction of the number of action potentials of individual nerve elements.

The concept auditory adaptation was suggested by de Maré in 1939 Up to the last few years, however the terminology has varied. All the following terms have been used interchangeably auditory fatigue and its German equivalent Ermüdung» (Békésy 1929 Schubert 1944 Hood 1950) physiological fatigue (Hood 1950) and in electrophysiological studies, equilibration (Derbyshire and Davis 1935)

Even though adaptation and fatigue may occur parallelly they should be clearly separated from each other Auditory fatigue follows intensive stimulation in the normal ear the maximum change taking place one half to one octave above the pure tone stimulus. The degree of fatigue increases continuously with the duration and the intensity of stimulation, or a balance is not reached until at abnormal sound intensities. Recovery is slow it is related to the degree of fatigue and, if the stimulus is strong enough, an irreversible change may result. Pure adaptation quickly attains a definite level in relation to the stimulus and there is no further increase the greatest change takes place at the stimulus frequency Recovery is rapid and does not depend appreciably upon the amount of adaptation (Zwislocki and Pirodda 1952, Kietz 1960, Zwislocki 1960 Hood 1960, Dishoeck 1954 1960)

The methods used for measuring human auditory adaptation are based mainly on changes in three psychophysical entities, i.e. on per and poststimulatory threshold shift, the decrease in loudness of suprathreshold stimulus, and decline of masked pure tone threshold as measured by an intermittent masking tone. Of these, perstimulatory threshold adaptation is the best known (Schubert 1944 Hood 1954 Palva 1956 a, b 1957 1961 Carhart 1957 Jerger et

al 1958 Jerger 1960 1962, Sorensen 1960 Palva and Palva 1961 1963) and it has also proved a practical test in the diagnosis of perceptive hearing impairments (Reger and Kos 1952 Johnson 1956 Sorensen 1962 a, Palva and Palva 1966 Palva et al 1967)

As regards perstimulatory suprathreshold adaptation in which the number of inner ear and auditory tract units involved in the hearing process is greater than at threshold level highly variable results have been recorded The other unadapted ear of the test subject has often been used as a reference without paying sufficient attention to the functional change caused by the comparison tone in the control ear (Wright 1960 Palva 1964) This source of error can be eliminated by using a suitable interrupted comparison tone, consisting of short impulses eliciting a response corresponding to normal even in the adapted ear (Hood 1950 1955 a, b) Because of this the adaptation of the control ear due to cross hearing of a strong stimulus becomes insignificant from the point of view of measurement, and on this basis the test can be applied reliably not only to the study of normal ears at high intensities but also to cases of unilateral hearing loss. The present study is limited to testing normal subjects by this method and to analysis of the various factors playing a part in the results.

II REVIEW OF THE LITERATURE

A. PSYCHOACOUSTIC MEASUREMENT OF ADAPTATION

1 ADAPTATION RECORDED AS A SHIFT OF AUDITORY THRESHOLD

The relationship between adaptation and auditory threshold can be studied either as a perstimulatory change by recording, as a function of time, the stimulus intensity required for obtaining the threshold, or it can be studied as the behaviour of threshold after stimulation. This latter method has been used to measure both the recovery after the slow phase of adaptation and, using a short stimulus, the rapid component of adaptation referred to in the literature variously as instantaneous adaptation (Lüscher and Zwislocki 1949) residual masking» (Munson and Gardner 1950) short duration auditory fatigue or adaptation (Harris et al 1951 Harris and Rawnsley 1953 a) initial auditory adaptation (Langenbeck 1960), forward mas king» (Raab 1961 Pollack 1964) or short-term temporal threshold shift (Ward 1963)

a) POSTSTIMULATORY THRESHOLD SHIFT OF SHORT DURATION

De Maré (1937), using a 0.4 sec. pure tone impulse, determined the threshold shift following a stimulus of equal duration, and somewhat later in his monograph (1939) using stimulation for 3 1 sec. at an SL (sensation level) of 44—88 dB The loss in threshold sensitivity occurred in a few tenths of a second and recovery was equally rapid. With increased duration and intensity of stimulation the threshold shift also increased but was followed by a decrease as the difference between stimulus and test frequencies became greater Later de Maré (1951) stated that the frequency distribution of the threshold loss is symmetrical around the stimulus frequency

Lüscher and Zwislocki (1946 1947 1949) found that recovery became complete within 250 msec. from a threshold shift of 40—50 dB produced by a 0.4 sec. 3000 Hz stimulus at 80 dB SL and measured by 20—50 msec. test tones. At the same sensation level, the degree of threshold shift was independent of frequency The amount of adaptation around the stimulus frequency corresponded in shape to the masking distribution, the main emphasis being on frequencies higher than the stimulus tone.

Harris et al (1951), Rawnsley and Harris (1952) and Harris and Rawnsley (1953 a, b) obtained similar results with the same testing values an exception, however was the frequency distribution of adaptation, which, as reported also by de Maré (1951), was symmetrical at sensation levels below 90 dB, extending to the higher frequencies with more intense stimulation — in agreement with Munson and Gardner s studies. In Zwislocki and Pirodda s report (1952) too, the maximum shift, with a 0 4 sec. 3150 Hz stimulus and

measured after 150 msec. interval with a 30 msec test tone, occurred at the same frequency at an SPL (sound pressure level) below 80 dB for 100 dB SPL stimuli however the maximum was half an octave higher This was considered by the authors a sign of incipient acoustic trauma or fatigue, similarly as by Munson and Gardner and Harris and Rawnsley (1953 a, b) Variations in stimulus duration (Zwislocki et al 1959) from 5 to 1000 msec. at 15—85 dB SL affected the amount of adaptation only slightly and recovery occurred in 150 msec., within which time the result was unaffected by the duration of the test tone

Sorensen (1962 b) used a 1000 msec. pure tone stimulus and measured the threshold after 50 msec. with a 30 msec. impulse having a frequency half an octave higher than the stimulus Adaptation was found for stimuli exceeding 50—55 dB SL, with increasing age at lower intensities at intensities 20 dB above incipient adaptation the threshold loss was 12—14 dB in all age groups

b) POSTSTIMULATORY THRESHOLD SHIFT OF LONG DURATION

The amount of threshold shift following stimuli shorter than 1000 msec. was found to correlate only with stimulus intensity and recovery was also very monotonous up to the fatigue limit, irrespective of intensity and duration of stimuli. A long term threshold shift, however depends not only on the frequency of the stimulus but also on its duration and intensity and there is a wide range of individual variations (Rawdon Smith 1935 1936 Ewing and Littler 1935) although in one and the same subject the change is very stable on repeated testing (Riach et al 1964) Ewing and Littler found the fatigue threshold to be high for 2 min stimuli from 55 to 105 dB SL according to the frequency (128—8192 Hz) used. Rawdon Smith also failed to obtain a change with 1—2 min stimuli below 80 dB SL at any one of the frequencies tested (400—4000 Hz). The discrepancy compared with later studies is explained by the slow threshold measurement technique in addition Ewing and Littler only regarded a shift of over 10 dB as significant.

Caussé and Chavasse (1942) on their part demonstrated a 3—4 dB decline immediately after a 30 dB SL pure tone stimulus of 40 sec. duration, the frequency ranging from 800 to 10.000 Hz but this decline did not occur below 600 Hz. The frequency distribution of the threshold was symmetrical with the stimulating frequency In Hood s (1950) studies, a 2048 Hz tone of 1 min duration at 50—90 dB SL caused a 8—10 dB threshold drop when measured with a continuous tone 10 sec. after cessation of stimulus, whereas this drop at 110 dB SL was already 60 dB The threshold shift produced at sensation levels above 80 dB with a 1000 Hz tone began to spread towards the higher frequencies and at 110 dB SL the maximum shift occurred at 1400 Hz. Hood referred to the threshold shift as poststimulatory fatigue and emphasized the difference between physiological and pathological fatigue after a given critical intensity limit, where the threshold shift began to increase rapidly extending towards the high frequencies while, at the same time recovery

slowed down At 2048 Hz the critical limit for a 1 min stimulus was 95 dB SL. Jerger (1956 1958) found the corresponding value for a 3000 Hz stimulus of 1 min. duration to be 95 dB SPL, the threshold being measured at 4000 Hz. The figure reported by Epstein and Schubert (1957) for 4000 Hz (3 min. exposure) was 80 dB SL, as appraised by the shift in the threshold and its maximum. Rüedi (1954) using white noise, found the corresponding limit to be 80—95 dB SL.

Davis et al. (1946, 1950) used intensive pure tone stimulation at 110—130 dB SPL, the stimuli varying in duration from 1 to 64 minutes the maximum shift then always occurred half an octave above the stimulation frequency The most effective stimulus tone turned out to be 4000 Hz recovery too, was always slowest at 4000 Hz irrespective of the stimulating frequency Average hearing loss as determined between one and one-half and nine minutes following the end of exposure over two-octave range from the stimulating frequency upward at an SPL of 130 dB was 65 dB for a 12 min. 4000 Hz stimulus, and correspondingly for 2000 and 1000 Hz stimuli 40 dB, and for 500 Hz 20 dB For a 60—65 dB threshold shift, an exposure of 32 min. was required at 2000 and 1000 Hz and 64 min. at 500 Hz. The above writers also called attention to changes during recovery in the quality of the test tone. No change occurred at the stimulus frequency at the frequency corresponding to the maximum threshold loss the pitch always sounded higher and a number of frequencies sounded the same.

Hood (1950) found that stimulus duration had no such critical limit as was the case with intensity the threshold shift increased linearly as a logarithm of time at 100 dB SL using 2048 Hz tones of 1—320 sec. duration The same conclusion was reached by Glorig et al. (1958 a, b c) with white noise at 100 dB SPL and by Ward et al. (1959) in noise bands of one octave at 80—100 dB sound pressure levels, the duration of stimuli varying from 1 to 100 min. (measured at 4000 Hz)

Recovery of the threshold shift is most rapid during the first few seconds after cessation of the stimulus and then continues at a slower rate (Hood 1950) For a 2048 Hz stimulus at sensation levels below 90 dB, recovery was complete in 1—2 min., but above 110 dB not even in 5 min According to Epstein and Schubert the threshold shift produced for 4000 Hz by a 3 min. sustained stimulus at sensation levels from 70 to 100 dB, when measured at the same frequency with a Békésy audiometer continuous tone was still about 10 dB in all cases 1 min. after cessation of stimulation Palva s (1958) corresponding results with the same technique after 20 dB SL stimuli were about 2 dB at 250 and 500 Hz, 6 dB at 1000 Hz, and 8—10 dB at 2000—6000 Hz. Using lower intensities (10—60 dB SL) and 1000 2000 and 4000 Hz tones, Bell and Fairbanks (1963) found a threshold shift of only 2 dB when starting threshold determination with a continuous test tone, a 60 sec. interval being allowed after the 1 min. stimulus. Ward (1960, 1963) stated that recovery from intensive noise stimulation occurred linearly as a logarithm of time after a 2 min interval, when the rapid phase of recovery was over

It should be stressed in this context that when measuring threshold with a continuous tone the result is modified by the adaptation caused by the test tone itself recovery is slower and the results higher than with an interrupted tone (Harris 1954 Kopra 1955 Bell and Fairbanks 1963 Bradford and Goetzinger 1964)

Hirsh and Ward (1951 1952) observed that recovery during the first two minutes consisted of two phases, when measured with short pure tone and noise impulses and using acoustic clicks after a 3 min pure tone and noise stimulation at an SPL of 100—120 dB The minimum in the curve occurred at 1 min and the second maximum or bounce 2 min after stimulation Hirsh and Bilger (1955) assumed that two independent components, R 1 and R 2 were involved while R 1 is complete in about 1 min and facilitatory in nature, R 2 occurs more slowly and regularly at a given rate and is related to the amount of threshold shift. In certain combinations there could be a transient sensitization of threshold as demonstrated by some investigators (Hughes 1954 Hughes and Rosenblith 1957 Rodda 1965) Hinchliffe (1957) expressed the view that the transition of a monotonous recovery curve into a polyphasic one is due not to adaptation alone but to the added fatigue effect too Rodda (1962 1965) also found these phenomena to be closely related to the transition periods and transition stimuli Without exception the observations made by other workers on the polyphasic recovery curve are all associated with intensive stimuli (Hirsh and Ward 1952 Hirsh and Bilger 1955 Jerger 1956)

Thus, poststimulatory threshold shift is affected primarily by the stimulating intensity and, above a given level, by additional changes to be considered fatigue phenomena, whereas below that level the change has been interpreted as associated with adaptation (Selters 1964) Selters first produced fatigue in one ear of the subject with loud noise, between 1700 and 2500 Hz, for periods of 4 min., followed by 1 min. tests until the sensitivity at 3000 Hz had been reduced by 35 dB After 50 min., when noise-produced threshold shift averaged 16 dB and ranged individually from 6 to 30 dB, he measured the additional change in the same ear at 3000 Hz for a 10 sec. stimulus using a 0.2 sec. test tone after an interval of 0 8 sec. and repeating the stimulus test tone in 11.5 sec. periods. The level of stimulation was 5 15 30 and 45 dB above TTS 50 min (temporary threshold shift 50 min. after primary stimulation) and 70 80, 90 and 100 dB above prestimulatory threshold The former threshold shift of long duration he attributed to the fatigue phenomenon, the latter solely to adaptation He compared the results with those obtained for 5—100 dB SL on the other ear The changes were directly additive, in other words fatigue in practice in regard to adaptation meant only a shift in stimulation level From this he concluded that the adaptation process is more centrally localized than the fatigue phenomenon Ward (1966) concurred in the opinion of Selters and, using the term intermediate TTS he stated that this (Selters s adaptation) possibly has the same localization as perstimulatory adaptation (Ward perstimulatory fat gue)

2. ADAPTATION MEASURED AS A CHANGE IN LOUDNESS

Determination of adaptation as a decline in loudness is based on measurement of the functional change in the stimulated ear by means of the other unadapted ear In this respect the method differs in principle from those described above and is physiologically more complicated owing to the involvement of interaural factors. This method offers another possibility of studying adaptation above threshold, in addition to the masking method (Thwing 1956 Feldmann 1958 a, b, c, 1960 a b 1962) which is associated with sources of error due to the long lasting measuring tone

On the basis of the characteristics of the control tone it is possible to classify a number of methods, all having three phases as a common feature The first consists of prestimulatory or initial balance during this phase the test subject adjusts the loudness of the tone presented to the control ear equal to the signal heard in the ear being tested, thus giving a plane from which the amount of adaptation can be calculated. During the adaptation phase proper an adapting stimulus is applied on the ear under test, and balances with the control ear are again made at fixed intervals. Recovery represents the third phase measurements are made at given intervals after the cessation of the adapting stimulus along the same principles as during the first phase.

a) POSTSTIMULATORY COMPARISON METHOD

As early as 1927 Pattie carried out tests measuring, after the initial balance with alternating pure tone impulses, adaptation after cessation of the stimulus by a comparison tone presented immediately to the other ear The intensity of the stimulus was 75—100 dB SPL and its duration 1—2 min The balances were obtained with 1 sec. impulses. The results expressed as a percentage (the prestimulatorily balanced comparison tone, when presented poststimulatorily to the other ear was then louder than the latter part of the stimulus) could be correlated with both duration and intensity of the stimulus and also be measured at frequencies other than that of the stimulus. Some of the test subjects made the balance in the perstimulatory phase, when fatigue — the term used by Pattie — was most distinct. Later (1929) he showed that the change was peripheral if one and the same tone was presented in different phases to each ear separately the tone localized to the ear with leading phase However there was no difference in adaptation between the ears, and if the tone was delivered only to one ear adaptation occurred on that side only

The same technique was used later by von Békésy (1929) Wood (1930), de Maré (1937 1939 1948) and in part of their studies, by Hood (1950), Egan and Thwing (1955) Kietz (1957) and Croce (1960). Von Békésy used comparison stimuli of 0.2 sec. duration arriving at the same conclusions as Pattie as regards stimulus intensity and duration He also found adaptation to be rapid and to vary widely from one individual to another Tests for the frequencies from

300 up to 8000 Hz yielded results which differed but little. Von Békésy noted further that following a pure tone stimulus, the tones lower than that stimulus appeared lower and the higher tones correspondingly higher than the comparison tone of equal frequency — an observation which he attributed to changes on the basilar membrane Owing to reduced sensibility of the nerve-endings in the stimulated area, he assumed these to respond more poorly to subsequent stimulation than did the more distant areas.

De Maré (1939) studied the change in loudness caused by a short stimulus, 2.7 sec. A pair of test tones, both of 0 4 sec. duration differing from the stimulus in frequency were then presented without interval one to the test ear and the other to the control ear The purpose of the change in frequency was to compensate the change in pitch due to adaptation and so to facilitate comparison With a stimulus of 512 Hz and a comparison tone of 482 Hz, the measured loss was 30 dB at the sensation level of 88 dB and for 2048 Hz stimulus, measured at 1098 Hz, the loss was 37 dB

If an interval of 0.2 sec. was allowed between the stimulus and the pair of test tones, adaptation decreased rapidly by about 20 dB Thereafter the decline was slower at an interval of 0.7 sec. the values were 6 dB (512 Hz) and 13 dB (2048 Hz) The corresponding observation was made by Hood (1950) who used alternate 0.3 sec. tones at intervals of 0 6 sec. (alternated binaural balance) at sensation levels ranging from 80 to 100 dB In his later investigation (1955 a b) with alternate 0.2 sec. stimuli adaptation could not be demonstrated although it was measurable even subsequently with longer (15 sec.) simultaneous tones Hood offered the explanation that an adapted end-organ delivered a full on-effect viz. a normal initial potential Egan and Thwing, using 0.5 sec. alternate 1000 Hz stimuli at an SPL of 80 dB confirmed this observation

b PERSTIMULATORY COMPARISON METHOD

If the same pure tone or noise is delivered simultaneously in phase to each ear of the test subject, he hears the combined phantom sound which is localized to one or the other ear or is within the head The localization of low frequency pure tones is influenced by both intensity and phase difference, that of high frequency tones by the former only According to Mills (1960) a divergence from the midline was caused at 1000 Hz and 50 dB SL, by a 1 dB difference in intensity the difference being slightly smaller at low frequencies but smallest (0 5 dB) at high frequencies.

Zwislocki and Feldman (1956) studied the effect of phase difference and found sensitivity to be about 2 for the low frequencies at medium intensity levels above 1300 Hz the effect of phase was no longer measurable.

In the case of white noise Egan and Thwing (1955) found that, when the noise n the two ears was in phase or 180 out of phase, loudness balance was made by localization of a phantom sound If phase relations were random

the balance represented comparison of the loudness of two separate tones. This was also the case if the components differed sufficiently in frequency the test subject then heard two separate tones (Egan 1955 a) Egan measured adaptation by the above two methods at 800 and 2000 Hz and there were no appreciable differences in results. Using one or the other of these methods, the following test modifications have been classified on the basis of the control tone variables (Wright 1960, Small 1963)

In the *tracking intensity method* the intensity of the control tone is varied continuously and the test subject adjusts it to the loudness required for balance This technique with two audiometers was first applied by Dix and his co-workers (1949) and later by Hood (1950), whose study is actually the first extensive investigation of perstimulatory adaptation at suprathreshold levels. Hood measured adaptation at three frequencies, 500 1000 and 2000 Hz, and at sensation levels ranging from 20 to 100 dB the comparison tone was usually sustained for 10 sec. and measurements of adaptation (fatigue») were made at 20 sec. intervals. The decline in loudness was gradual and reached a maximum in 3 min. at less than 80 dB level. At 1000 Hz and 80 dB SL adaptation was 35 dB It grew with an increase in intensity and frequency An interesting observation concerning the physiological background of this phenomenon was that adaptation for a given group of receptors was always the same, regardless of the stimulating intensity i.e when the adaptation caused by a 1000 Hz stimulus at sensation levels of 20 40 60, 80 and 100 dB was measured in each case at 20 dB SL, it was invariably the same, 5—10 dB. Hood measured recovery starting continuous balancing at varying intervals after cessation of the stimulus.

Kunze s (1950) technique was similar apart from the fact that he performed the balance in a very brief (kurzfristig) period at intervals of 5 or 10 sec. on the basis of the localization of phantom sound. At the sensation level of 60 dB, adaptation in the frequency range 125—4000 Hz was always about 20 dB under 2 min. stimulation in which time the maximum could be reached

Palva (1955) studied the sensation levels of 60 and 80 dB at 500—4000 Hz using the *same* method on *normal subjects* and on *patients with various groups* of hearing impairment The same technique was used by Small and Minifie (1961) in studying the effect of the on and off time of the control tone on the amount of measured adaptation, and by Sergeant and Harris (1963) who investigated the relations between the tests for adaptation. Tsuiki s (1965) modification was similar in principle but differed from the preceding in one point: the control tone was interrupted (repetition period 2500 msec. — no other values are given) and was sustained for the entire duration of adaptation period Harbert et al (1966) have used the same technique with on-time of 350 msec. and off time of 650 msec. They obtained 13 dB adaptation at 80 dB SPL for 800 Hz stimulus.

Hood s technique modified was used by Pestalozza (1953) Manzini et al (1956) Mantegazzini et al. (1956) Bosatra (1957) Cioce and Pestalozza (1958 1960) Bocca and Pestalozza (1959) Pestalozza and Cioce (1962) and Cioce

and Spelta (1963) In Pestalozza s modification the stimuli in prestimulatory (initial) balance were frequently interrupted to avoid adaptation and the adaptation (final balance) was measured with a 5—10 sec comparison tone. Normal adaptation was defined as a 30 % loudness level decline in 3 min. at 50 dB SL in the frequency range 1000—4000 Hz.

The *method of fixed intensity* differs from the above only as regards the way in which the control tone is varied, the adapting stimulus being maintained in both at constant intensity level In this method only one balance is performed during each presentation of the comparison tone. Egan (1955 a b) Egan and Thwing (1955), Thwing (1955) and Jerger (1957) used this technique varying the intensity of the continuous comparison tone in 2 dB steps the duration of a balance was usually 15 sec. at intervals of 45 sec and the balance was always started below the final loudness level The balance was made on the basis of both median plane localization of the phantom sound and equality of loudness of the components. The resulting figures were lower than Hood s (1950) and the maximum adaptation did not occur until at 6—8 min Thwing (1955) also studied the frequency distribution of adaptation at 1000 Hz using a stimulus of 80 dB SPL, reporting it to be almost symmetrical (range 100—2500 Hz) with the stimulating frequency Jerger (1957) called attention to the amount of individual variation in adaptation, obtaining for a 1000 Hz stimulus at an SPL of 70 dB a range of 5—45 dB Wright s (1959) results with the same technique agree well with those of the preceding investigators. In white noise of 60 dB SPL, adaptation measured for a 4000 Hz pure tone at 90 dB SPL was 5—10 dB greater than in the absence of noise.

Wittich (1966) measured adaptation on the basis of median plane localization varying a 190 msec comparison tone in 1 dB or 5 dB steps, the stimulus-control tone combination being repeated after a pause which permits full recovery Using this modification adaptation reached a maximum, at 33—73 dB SPL and 2000 Hz, in 16 seconds and then remained unchanged for the longest stimulus tested, i.e. 5 min. At an SPL of 73 dB adaptation was 24 dB

The *method of varied intensity* was developed by Egan (1955 a) He started from the assumption that balance could be influenced by the formation of a subjective loudness standard to eliminate this, he either reduced or increased the stimulus intensity for the duration of balance Thus measured, the adaptation values were greater than when using the method of fixed intensity

For 800 Hz tones at 80 dB SPL the maximum adaptation values reached with the respective methods were 27 dB and 17 dB Egan and Thwing employed the same modification in prestimulatory balancing, and so did Carterette (1956) in his studies dealing with adaptation for noise.

In the *method of moving phantom* the criterion for adaptation is the time required for the phantom sound to move from the control ear to the median plane, the other stimulated ear being fully adapted (Wright 1960 1963) Wright s results indicate that, on stimulation of one ear for 7 min. at 80 dB SPL with a 4000 Hz tone, 25—50 sec. were required for an identical comparison stimulus to reach the same adaptation level, but only 0—10 sec. when using a comparison

tone at an SPL of 40 dB Conversely this means that the adaptation caused by primary stimulation is about 40 dB

) INFLUENCE OF STIMULUS AND COMPARISON TONE VARIABLES ON RESULTS

The adaptation obtained by various experimenters seems to differ widely even when using the same balancing time, mostly a 15 sec. sustained tone Thus, for instance, as pointed out by Wright (1960) and Small (1963) Hood (1950) varying the intensity level of the comparison tone rapidly reported a considerably greater adaptation than Palva (1955) who used a rate of 2.3 dB/sec. Hood's figures for a 3 min. sustained 1000 Hz stimulus at 80 dB SL were 30—35 dB, Palva's corresponding figures were generally below 10 dB for stimuli between 500 and 4000 Hz. The figures given by Small and Minifie (1961) using an attenuation rate of 5 dB/sec., are intermediate between Hood's and Palva's adaptation after 6 min. stimulation at 4000 Hz and 75 dB SL was 20—25 dB when using the time variables corresponding to the control tone of the latter two. The result is of the same order as those obtained by Egan (1955 a) Egan and Thwing (1955) Thwing (1955), Jerger (1957) and Wright (1959) with the fixed intensity technique, the attenuation occurring in 2 dB steps and the rate being adjusted by the test subject.

It is obvious, because of the quick development of adaptation, that even a 15 sec. control tone must change in loudness during its presentation. If one uses a slow rate for intensity change of the control tone, its average level stays higher than when the change is rapid, the balancing being started in each case at the same prestimulatory level. This also implies that, in the former case the adaptation to the control tone is greater and the source of error therefore more marked, which accounts in part for the differences among the results.

Small and Minifie studied adaptation in the comparison ear by varying the duration of the control tone and the tone interval, in 10—50 sec. limits, using a 4000 Hz stimulus at a sensation level of 75 dB If the interval exceeded 30 sec., the result was not affected by varying the control tones from 10 to 30 sec.: maximum adaptation, 20—25 dB occurred at 5—6 min If the interval was shorter less adaptation was measured — thus indicating a cumulative adaptation in the control ear

A special study of the errors arising with a 15 sec. continuous comparison tone was undertaken by Wright (1960) He started balancing, based on localization of the phantom sound, either 15 dB above or 15 dB below the intensity of the stimulus (250 1000 and 4000 Hz pure tones) The results over a 15 sec. run differed slightly from one another which indicated different adaptation in the control ear depending on the condition of test. Secondly he stated that there is rapid adaptation when measured by the moving phantom method. Thirdly he showed, using 1 sec. control tones, that adaptation reached 50 dB in 7 minutes for a 500 Hz 90 dB SPL stimulus, whereas Egan's (1955 a) and Jerger's (1957) corresponding value was as low as 20 dB with a 15 sec.

continuous balance. Kunze (1950) used a very short balancing time and obtained 40 dB adaptation for a 500 Hz pure tone at a sensation level of 80 dB Tanner's (1955) results with 2—4 sec comparison tones were lower corresponding to those of Hood (1950)

Croce (1960) compared the technique of Hood with Wood's (1930) poststimulatory balance carried out with a 5 sec. test tone for a 3 min 50 dB SL stimulus he obtained about 7 dB more adaptation at 1000 and 2000 Hz, i.e 16—17 dB, with the former method

Measured with short-duration comparison tones, maximum adaptation seems to be reached earlier than with long tones. Tanner obtained maximum in 2—4 min., Tsuiki (1964) in 1—3 min Wittich (1966), who used a 2000 Hz stimulus and a single 190 msec. control tone in a repeated sequence obtained maximum adaptation in 16 sec. at 33—73 dB SPL. With Jerger's (1957) technique adaptation increased up to 7 min., except for frequencies at and below 500 Hz, which showed a maximum at 3 minutes. The corresponding results of Wright (1959) are in agreement with the above Investigators using a similar technique also included Carterette (1955) who found that adaptation to noise increased up to 7 min in the case of a 50 dB SPL stimulus but the maximum appeared in 1 min. when measured at lower intensities

Independently of the measuring technique, investigators agree in principle about the relationship of the stimulus variables to adaptation As reported by Jerger (1957) adaptation increases progressively as a function of frequency in the range 125—1000 Hz, remaining then fairly constant up to 8000 Hz, the tests were made at an SPL of 90 dB The results of Hood (1950) and Wright (1959) support this finding Jerger (1957) expressed adaptation in terms of log. adaptation in sones the differences between frequencies were thus evened out and the curve as a function of loudness level (dB) was straight.

Wright (1959) measured adaptation to a 90 dB SPL pure tone under masking with white noise of 60 dB SPL (125—8000 Hz) Adaptation to a 4000 Hz pure tone both at 1 min. and at its maximum was higher than in the absence of noise there was no cumulative effect at 250 and 1000 Hz, which shows that the effect of noise differs at the various frequencies tested

Adaptation also increases with the stimulus intensity According to Jerger (1957) the increase was roughly linear with the sensation level up to 60 dB after which it was more slow Tested by Hood's (1950) technique, the increase was nearly linear up to the sensation level of 100 dB By the same technique as modified by Tsuiki, adaptation did not increase further above the sensation level of 60 dB and after 85 dB there was rather a decrease. Wright (1956) obtained practically the same adaptation at 85 dB SL as at 55 dB Correspondingly the values reported by Palva (1955) are essentially the same at 80 dB as at 60 dB SL.

Measurement of recovery from adaptation is a special problem The result is decisively affected by the duration of the stimuli used If a tone of long duration is used, it as such causes added adaptation which delays recovery

If however short stimuli are applied no adaptation whatsoever can be measured (Hood 1950, 1955 a, b, Egan and Thwing 1955) Hood stated that the adapted end-organ produces a full on-effect. Wittich measured the recovery with simultaneous 190 msec. stimuli at 2000 Hz after the maximum adaptation reached by means of stimuli at sound pressure levels of 53 dB and 73 dB Poststimulatory at 20 msec. adaptation was in each case 12 dB the respective maxima being 14 and 24 dB After the longest interval studied, at 160 msec., the values were respectively 5—6 dB and 6—7 dB

In general, the measurements of recovery have been made along the same principles as the initial balance. When the technique of Thwing (1955) was used, the recovery of adaptation was often complete in 2—3 min. following stimuli at sound pressure levels of 60—90 dB Wright (1959) on the other hand, found that 3 min. after a 90 dB SPL stimulus (250 1000 4000 Hz) there was still on an average a 3 dB residual adaptation According to Hood (1950) recovery after a 1000 Hz 80 dB SL stimulus lasting 3 min became complete in 60 sec. Carterette (1955 1956) reported complete recovery of adaptation for white noise within 4 min. following 7 min. stimuli at sound pressure levels ranging from 30 to 100 dB Recovery seems to be very rapid for the first few seconds and then to slow down.

3 INFLUENCE OF CONTRALATERAL STIMULATION ON ADAPTATION

The effect of contralateral stimulation may also appear during tests for TTS thus the pure tone poststimulatory threshold shift became smaller and the recovery period shorter as a result of perstimulatory contralateral low-intensity noise (Maspetiol et al. 1961 Pennetta and Pinto 1962, Burghoff 1962, 1963) Ward (1965) compared binaural pure tone and noise stimulation (115—125 dB SPL) with monaural stimulation obtaining about 5 dB lower values with the former he explained that the difference occurred on the basis of the stapedius reflex but that the part played by the efferent auditory bundles could not be excluded Hirsh (1957 1958), who used 110 dB noise stimuli and 20—80 dB 1000 Hz pure tone stimuli, did not demonstrate any significant difference between binaural and monaural stimulation

Hahn and de Michelis (1960) studied the effect exerted on perstimulatory adaptation at threshold level by continuous and interrupted (frequency 1/sec.) white noise at sensation levels of 15 and 30 dB In test subjects who heard the test tone at threshold for 60 sec., the introduction of contralateral noise at 30 dB level reduced the duration of sensation to 15—20 sec and intensity had to be increased by 10 dB for the sensation period to be 60 sec. The effect of interrupted noise was less marked. However those test subjects in whom the sensation period at threshold was shorter so that a further 5—10 dB was required for 60 sec. to be attained, showed the opposite result the sensation period at threshold was prolonged and the added intensity reduced to 0—5 dB Brunetti (1960, 1961) Hahn (1962, 1963) and Maspetiol and his co-workers (1962) used an identical technique but usually obtained 5—10 dB more adaptation in 1 min.

It was stated by Collins and Capps (1965) and Capps and Collins (1965) that mental activity viz. mental arithmetic, increased the poststimulatory threshold shift by 5 dB after a 40 dB SL stimulus. Wernic and Tobias (1963) reported the corresponding value to be 2—4 dB increasing to 10 dB for stimulation at 90 dB SL. According to Hahn and de Michelis an intermitting light stimulus (frequency 20/sec.) caused a 5—15 dB increase in perstimulatory threshold adaptation in 60 sec The effect was less at lower frequency rates (2/sec. and 4/sec.) Activity in the form of mental arithmetic caused adaptation to decrease in the group in which contralateral tone stimulation also reduced adaptation whereas increased adaptation resulted in the group in which a contralateral stimulus also increased the adaptation The change resulting from the use of light stimulus was assumed to be a response of the bulbomesencephalic plane while in the case of mental arithmetic there was thought to be a cortical effect.

Price and Oatman (1967) made a control study on the work of Wernic and Tobias, concluding that it may be a question of artifacts. If the test subject in the final stage concentrated strictly on threshold determination the difference disappeared Bell and Stern (1964) also were unable to confirm the results of the former investigators

B ELECTROPHYSIOLOGICAL BACKGROUND OF ADAPTATION

The literature dealing with the electrophysiology of hearing is very extensive Only the main findings will be considered here, i.e. those contributing to better understanding of the adaptation phenomenon It should be noted, however that there may not be a direct correlation between the results of electrophysiological tests on animals and of psychophysical measurements on the human ear

Cochlear microphonics (CM) represent very closely the electrical equivalent of sound energy up to a given intensity (Davis et al. 1958) after which follows a decrease interpreted as being due to fatigue For the guinea pig the transitional limit at 500—2000 Hz was 95 dB re human threshold (Gisselsson and Sorensen 1959) for the cat it was 75—90 dB at 250—3000 Hz (Hughson and Witting 1934) and 100 dB at 5000 Hz (Burgeat and Burgeat Menguy 1964) The results thus correspond well with the fatigue limits of the human ear At lower intensities CM are characterized by the fact that they do not change as a function of time there is no adaptation.

Adaptation occurs, however in the action potentials of the auditory nerve (Derbyshire and Davis 1935) There is a fast equilibration complete within 2 seconds or less and there is in addition the familiar slow equilibration, which is complete in about 7 minutes. Recovery requires about 30 seconds. No adaptation occurred in the cochlear potentials registered from the round window

The response of single auditory-nerve fibres was measured by Galambos and Davis (1943) The auditory fibre responded to a continuous adequate stimulus

by a train of impulses which were initially numerous but declined rapidly (rate-adaptation), adaptation being complete in a few tenths of a second. The amplitude of the action potentials also diminished to some extent.

At the level of the superior olivary complex, adaptation to a 10 sec pure tone appeared as a decline of the firing rate but not as a change in the number of activated neurons (Goldberg et al. 1964).

In the inferior colliculi the response to white noise was slower than that of the cochlear nucleus (Thurlow et al. 1951) but in most cases response dropped practically to zero in a few seconds. A slow electrical component, such as that measured by Galambos (1952) from the medial geniculate body also became evident and it remained unchanged during 15 min. stimulation.

The recovery of the auditory cortex (Rosenblith et al. 1950) occurred parallel to the first neural component measured from the cochlea, the test tone being a click of 0 1 sec. duration and the stimulus a 500 Hz tone at 115 dB SL sustained for 60 sec. According to Keidel (1958), there was adaptation when measured from the auditory cortex with a series of clicks (frequency 10/sec.) even though no such response was obtained from the auditory nerve

The efferent auditory bundles have been found to affect cochlear function and these findings shed new light on the adaptation problem

The crossed efferent olivo-cochlear bundle described by Rasmussen (1942 1953 a, b,) the uncrossed olivo-cochlear bundle, and the direct efferent bundle from the reticular formation to the cochlea (Rossi and Cortesina 1965) constitute the peripheral part of the efferent system originating from the central nervous system. Electrical stimulation of the efferent bundle resulted in a decrease of the action potentials of the afferent bundle (Galambos 1955 1956 a, Desmedt and Mechelse 1958 and Sohmer 1965) Fex (1959 1962) and Sohmer advanced the opinion that a decrease in action potentials in the cat was associated with a slight rise in cochlear microphonics. In addition a slow positive potential possibly derived from the efferent endings was measured from the round window (Fex 1962, 1967) A change corresponding to the drop in action potentials of the auditory nerve was also found in all nuclei of the afferent tract (Ruben and Secula 1960, Desmedt 1962).

Fex (1962, 1963) and Pfalz (1962 a, b c, 1966) studied the activity set up in the olivo-cochlear pathways by a tone stimulus. They found that most of the neurons of both the direct and the crossed bundle were characterized by a well-defined best frequency by a definitely fixed threshold and regular function. Fex stated that, the crossed bundle responded mainly to stimulation of the ear into which it passed, the direct bundle responded to contralateral stimulation.

The efferent acoustic bundles are not important only in the formation of acoustic contrast (Pfalz 1962 a) in listening (Galambos 1956 b) and acoustic habituation (Hernández Péon 1955 Hernàndez Péon et al. 1956 1957 Galambos et al. 1956) the entire efferent nervous system also controls the information reaching consciousness (Keidel 1966)

Leibbrandt (1964, 1965) made interesting observations as regards the relations between the efferent bundles and adaptation When action potentials were measured from the round window in the guinea pig, there was normally adaptation which was complete in 75—100 msec., the stimulus consisting of 2 msec impulses at 10 msec. intervals and the sound pressure level being 60 dB at 5000 Hz. If procaine was injected into the internal auditory meatus, the drop in action potentials failed to occur He considered the failure of adaptation due to blocking of the efferent bundles.

III AIMS OF THE INVESTIGATION

This investigation was designed to study normal-hearing subjects, under conditions as far as possible free from sources of error to determine the perstimulatory decline in loudness level of a continuous pure tone stimulus at suprathreshold levels using a binaural balancing method. Attention was directed to the following points

— the amount of adaptation as a function of sensation level, frequency and time

— the relations between adaptation and age of the test subject

— the changes in sound quality associated with decline in loudness level of the adapting stimulus

— the prestimulatory balance related to various sensation levels and frequencies

— the changes in excursion width of the tracings obtained with a self recording audiometer during adaptation measurement as compared with threshold recording and prestimulatory balance

— the recovery of the ear after pure tone stimulation based on threshold recording

The test was developed with a view to its application to the study of hearing impairments.

IV APPARATUS MATERIAL AND METHODS OF INVESTIGATION

A APPARATUS

The principle of the testing equipment is presented in Figure 1 A Grason Stadler Model E800 Békésy Audiometer was the main unit The interrupted test tones used were those of the audiometer pure tone channel (channel 1) and their intensity was adjusted by the test subject by means of the subject switch The rate of the intensity change either 2 1 or 4.2 dB/sec., in 0.25 dB steps could be varied by the operator In addition to the regular attenuator the operator also had a ± 20 dB fixed attenuator at his disposal

The continuous pure tones were fed from an external sound source (Philips Tongenerator GM 238) into the Grason Stadler audiometer channel 2 through the masking external input jack The pure tone stimulus selected was calibrated with the audiometer voltmeter for each frequency on each occasion of test, and the Grason Stadler audiometer attenuators alone were used for intensity control. The stimuli were delivered to the earphone either continuous or interrupted with the aid of an electronic switch The audiometer allowed the sound pressure level of channel 2 to be adjusted by the operator in 5 dB steps.

The channels were connected to the earphones by means of a switch box with which the test tone could be switched from one ear to the other The Grason Stadler M20L42 earphone was used on channel 1 and M11L35 on channel 2, and they were mounted in MX41/AR cushions and attached to a light weight headband Calibration of the audiometer was checked with the artificial ear (coupler A 9) in the usual fashion

The tests were made in the Audiologic Laboratory of the Otolaryngological Department of Oulu University The apparatus and the test subject were in separate soundproof rooms, the operator having a view of the test subject through a 3-glass window

Background noise measured (Precision Sound Level Meter Type 2203 Brüel and Kjaer Denmark) at the test subject's position was less than 45 dB SPL re 0 0002 dyn/cm² Measured by octave filter (Type 1613 Brüel and Kjaer) the noise proved to consist mainly of frequencies below 250 Hz. In octave bands at the mean frequencies 250 500 1000 2000 4000 and 8000 Hz, the attenuation rate being about 45—50 dB/octave, the noise never rose above 25 dB SPL re 0 0002 dyn/cm²

Madsen Model OB 60 audiometer (NPL standard) was used to determine hearing thresholds by the descending ascending method in selecting test subjects.

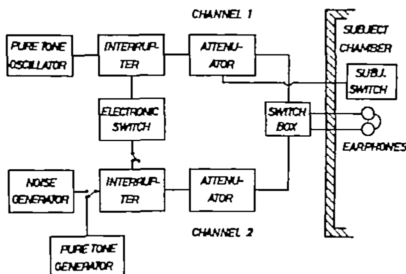


Fig. 1 Block diagram of testing equipment. (For explanation see text).

B MATERIAL AND TECHNIQUE OF TESTING

The group termed normal consisted of 38 healthy test subjects with normal hearing they ranged from 20 to 45 years in range with an average of 25.4 years. Two-thirds were medical students, the rest nurses, or other hospital staff Three of the test subjects had previous experience of auditory testing. Those admitted to the series were free from any ear diseases and were also otherwise healthy Air conduction hearing was better than 10 dB at the studied seven frequencies re normal threshold (NPL standard)

It became abundantly clear during the progress of the study that adaptation in children differs from that recorded in adults, and measurements were therefore made, after preliminary tests, on 29 subjects aged from 7 to 15 years (average 12.0 years) This group was selected among patients seen at the Otolaryngological Outpatient Clinic. All of these attended the Clinic for recurrent tonsillitis but were healthy on the occasion of test: the tympanic membranes were of normal appearance and air conduction hearing was within 10 dB of the normal threshold at the frequencies 125 250 500 1000, 2000 4000 6000 and 8000 Hz.

In addition, tests were made on 10 subjects aged 56–70 years (average 60.0 years) One half of these were healthy old people who had been specially asked to present themselves for the tests, the other half consisted of patients earlier treated at the Otolaryngological Clinic none of these had a history of ea disease and all had normal drum membranes. Air conduction hearing for these ears individually appears in Figure 2. Taking into account the age of

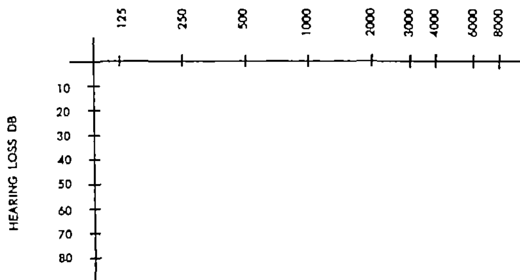


Fig. 2. Individual air conduction values (NPL-standard) obtained for test ear of 56—75 yea old subjects.

each subject studied, the values are consistent with those reported in presbycusis (Staloff and Menduke 1957)

Adaptation was measured in one ear of each subject. The normal group included 16 right and 22 left ears, the children correspondigly 14 right and 15 left ears, and the old age group 7 right and 3 left ears The normal group consisted of 20 women and 18 men, the children's group of 11 girls and 18 boys, and the old age group of 7 women and 3 men

In the normal group each subject was tested for the frequencies 250 500 1000 2000 3000 4000 and 6000 Hz at the sensation levels of 20 40 60 and 80 dB Twenty-eight subjects were tested at each of these levels, the remaining 10 at one to three levels, in such a way that all four sensation levels were studied on 32 ears. The frequencies were so selected as to enable application of the test to the study of hearing impairments.

Each subject was only tested at one sensation level during one and the same day the testing order being from the highest frequency 6000 Hz, downwards, to exclude the fatigue spreading chiefly to frequencies higher than the stimulus. To demonstrate possible fatigue effect, 27 subjects were also tested at 60 dB SL from the lowest frequency upwards. Tests at the various sensation levels were carried out in arbitrary order

The measuring technique requires sustained concentration and it appeared that the interest and patience of the youngest subjects sufficed for only one or two recordings for this reason the tests in this group were limited to 2000 and 4000 Hz on the basis of experience gained in the normal group as regards ease of comparison and amount of adaptation In the old age

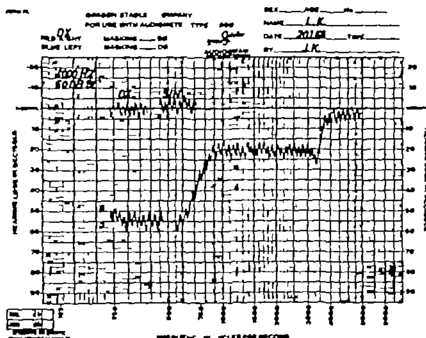
group the measurements were made at only one sensation level, 60 dB, for seven frequencies, at 250 500 1000 2000, 3000 4000 and 6000 Hz.

Both the threshold and adaptation tracings were registered at sound pressure levels re 0.0002 dyn/cm² (channel 2 basic level), the rate of attenuation being 2.1 dB/sec. in 0.25 dB steps In two subjects adaptation developed so quickly that the audiometer rate 2.1 or 4.2 dB/sec. was not sufficient to keep up with adaptation during the first seconds. By means of the plus-minus 20 dB switch, the intensity of the comparison tone could be abruptly changed by 20 or 40 dB It proved difficult, however for the test subjects to obtain a balance during the first seconds even by this means so it was decided, in these cases too, to use only a 2 1 dB/sec. attenuation rate for greater consistency of testing

The durations of the interrupted pulse and of the pulse interval were 200 msec. at a rise-and fall time of 25 msec., sufficient to eliminate switching transients. It was decided to use this comparison tone because it had proved ideal from the point of view of measurement of adaptation, an added advantage being that this signal is available on the Grason-Stadler Model E800 audiometer and the test is thus easily applicable.

At the beginning of testing the subject was familiarized with the self-recording audiometer by letting him determine threshold values for each ear at a fixed frequency he was instructed to press the switch immediately he hears a pulsed tone and release it as soon as the tone disappears. For every frequency tested the subject, on each test occasion, determined first the thresholds for the two ears, using an interrupted signal and intensity bein, varied at a rate of 2.1 dB/sec. This threshold determination took on an average 60 sec. The operator then, using channel 2, measured once more the threshold for the experimental ear by the descending-ascending method with a pulsed signal the value obtained was taken as a basis for calculation of the sensation level.

This was followed by prestimulatory balancing on channel 2 an interrupted stimulus at the desired sensation level was presented to the ear under test and at the same time a comparison tone of the same frequency to the other ear the pulses being simultaneous. The test subject was asked to adjust the intensity of the comparison tone until its loudness equalled that of the stimulus, in other words to keep the switch in the up position as long as the comparison tone was softer than the stimulus and to press the switch down as soon as the comparison tone appeared louder thus trying continuously to keep loudness as closely matched as possible. When the test subject attained the level of balance and recorded it for 20–30 sec., in some cases for 60 sec., both signals were discontinued for a few seconds. An adapting, i.e. a continuous, stimulus was then introduced on the experimental ear for 3 min., or longer f adaptation continued to increase. Balancing with an interrupted tone was continued throughout the stimulation along the same principle as in prestimulatory balance. The subject was requested to pay attention also to changes in sound quality besides to possible loudness change



F g. 3 Perstimulatory suprathreshold adaptation measured on right ear t 60 dB SL 4000 Hz. Above on left are shown the threshold tracings for each ear and below the prestimulatory balance tracing. During a pause of a few second the operator has changed the position of the recording chart; in the middle is seen the intensity decline the comparison ear during 3 min. requiri g for loudness balance. The recovery f the poststimulatory threshold for the right ear ppe rs at the extreme right

Immediately on cessation of stimulation an interrupted channel 1 tone was presented to the experimental ear by means of the switch box. The test subject recorded the recovery of threshold for 1–2 min at the stimulus frequency

Figure 3 shows the result of a typical test run at 4000 Hz and 60 dB SL. Testing at one intensity level thus required on average 60–70 min., the intervals between adapting stimuli being at least 5 min

C. STATISTICAL TREATMENT

For the calculations, the mid points of each amplitude of the threshold, prestimulatory balance and adaption tracings were determined and the average curves were drawn through these points. The prestimulatory balance level was expressed as the difference between the 20–30 sec. average and the threshold in the control ear or in some cases in which the tracings showed continuous fluctuation between the 60 sec. average and the control ear threshold Adaptation was determined in terms of decibels of the prestimulatory balance level in other words as the difference between the adaptation curve at 15 30 45 60 90 120 150 and 180 sec. and the prestimulatory balance level To determine the points in time a graduated ruler was prepared on the basis of twenty accurately timed tracings

The amplitudes of all the tracings were determined, interpolating the excursion maxima and minima for a period of 15—30 sec., at a point where the tracing was free from errors. For the adaptation tracing it was determined both during the first minute and at the end of the curve i.e. when adaptation was increasing and when it was fully developed. Post stimulatory threshold amplitudes were calculated at the end of the first minute.

The results were dealt with in the Computer Centre of Oulu University Statistical analysis was made somewhat difficult by the skewness of the distributions and their wide variability However the distributions were taken as normal distributions and the means compared by the t-test. The differences between the means were considered significant if the probability of error was $P \leq 0.05$

For comparison nonparametric tests were also used in some cases the distribution then need not be assumed to be normal. The results in all these cases were consistent with those obtained by the t test.

V RESULTS

The testing technique, especially continuous loudness balance, requires sustained concentration on the part of the test subject, and also some skill in adjusting the audiometer It appeared however that even a child of 7–8 years if co-operative acquired the technique easily and could perform the test in every respect successfully Exceptions were a few timid children and the test in their cases had to be discontinued because even threshold recordings failed In the group of adults, the recording was always successful. The values for one subject however were omitted in calculating the results because of difficulties in interpretation of the tracings. Despite repeated instructions, this subject tended to make the balance with very small excursions and the tracing fell continuously the level being corrected by the subject at intervals in wide steps

A NORMAL ADULT GROUP

The results given represent means and standard deviations (S D) for 32 test subjects. An exception was the sensation level of 80 dB at 250 Hz only 27 subjects were tested at this intensity owing to technical difficulties at the outset

1 PRESTIMULATORY BALANCE

Balance level

On reaching balance, most of the subjects kept this level stable within the limits of 0–5 dB In five ears, the tracing almost regularly fluctuated continuously but there were no excursions beyond ±5 dB in any of the frequency SL combinations, and in these 5 cases the balance was recorded for 60 seconds.

Balancing could vary considerably from one subject to another but it was characteristic that in the case of one and the same subject, the balance at all sensation levels and frequencies was as a rule either higher or lower as compared to the averages. The results are presented in Table 1 The balance levels differed statistically significantly at all frequencies. Taking all sensation levels into account, they seemed to be higher for the medium frequencies than for the lower and higher frequencies. The differences were significant in the following combinations 40 dB SL 500 and 1000 Hz v 6000 Hz 60 dB SL 500 Hz v 3000 Hz 80 dB SL 500 Hz v 250

TABLE 1

Prestimulatory balance levels

Frequency (Hz)	Sensation level (dB) 20 Mean	S.D	40 Mean	S.D	60 Mean	S.D	80 Mean	S.D
250	20.8	5.6	40.5	7.8	57.7	8.7	75.2	9.2
500	21.2	6.6	41.6	7.8	61.3	8.8	80.0	8.1
1000	21.8	5.0	41.0	8.3	58.9	10.0	78.6	8.5
2000	20.9	6.6	40.6	8.4	57.4	11.4	75.3	11.1
3000	21.8	6.9	37.9	8.0	56.6	6.9	76.1	8.4
4000	21.2	5.6	37.9	8.1	57.1	8.2	76.5	8.9
6000	19.5	5.7	36.9	7.3	58.0	8.8	75.2	7.3

and 6000 Hz. With an increase in sensation level, especially at 60 and 80 dB SL, the balance level tended to decrease as compared with sensation level. At 80 dB SL, the difference between these levels was significant for the frequencies 250 2000 3000 4000 and 6000 Hz, at 60 dB for 3000 Hz, and at 40 dB for 600 Hz.

TABLE 2

Prestimulatory balance amplitudes (dB) compared with threshold amplitudes of control ear

Frequency (Hz)	Sensation level (dB) 20 Thr Mean	Thr S.D	Bal. Mean	Bal. S.D	40 Thr Mean	Thr S.D	Bal. Mean	Bal. S.D	60 Thr Mean	Thr S.D.	Bal. Mean	Bal. S.D.	80 Thr Mean	Thr S.D.	Bal. Mean	Bal. S.D
250	6.6	2.4	7.1	2.8	6.6	1.9	7.3	2.8	6.0	1.8	6.9	3.0	5.8	1.8	6.4	2.2
500	6.0	2.1	6.9	3.1	6.3	1.9	7.1	2.5	5.6	1.8	6.3	2.2	6.0	1.9	6.3	2.6
1000	5.9	2.1	7.0	2.8	6.0	1.8	7.1	2.9	5.6	1.9	6.3	2.2	5.5	1.7	6.5	2.6
2000	5.8	1.7	7.2	2.5	5.6	1.8	7.3	2.8	5.2	1.7	6.6	2.3	5.3	1.4	6.8	3.1
3000	5.7	1.8	7.6	3.2	5.8	1.5	7.2	2.3	5.5	1.7	6.2	1.7	5.4	1.7	6.7	2.4
4000	5.8	1.8	7.3	2.4	5.6	1.7	7.0	2.5	5.0	1.2	6.6	3.1	5.2	1.3	6.2	2.4
6000	5.4	1.4	7.9	3.1	5.3	1.6	6.9	2.2	5.0	1.1	6.3	2.3	4.9	1.3	5.9	1.8

Thr = Threshold
Bal. = Prestimulatory balance
= Difference between means significant ($P \leq 0.05$)

Balance amplitudes

The widths of the balance amplitudes are compared in Table 2 and Figure 4 with those of the control ear threshold recorded before the prestimulatory balance on each occasion of test. The balance tracings were significantly greater at all frequencies except 250, 500 and 1000 Hz, at which the threshold amplitudes tended to be greater than at the other frequencies. All frequencies considered, the minimum and maximum threshold amplitudes were 2 dB and 15 dB respectively those of the balance tracings correspondingly 2 dB

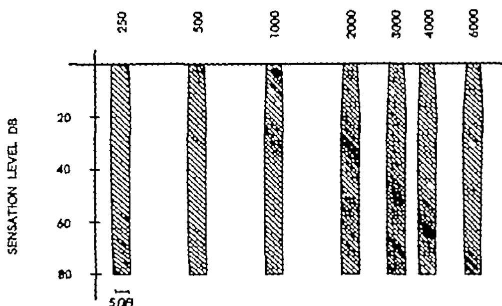


Fig. 4 Excursion widths of prestimulatory balance trancings compared with threshold amplitudes of the comparison ear

and 20 dB most of the values in both groups ranged from 5 to 10 dB The difference concerned is evidenced as greater dispersions of the balance amplitudes in Table 2.

At the threshold level the tracings showed a tendency to decrease with an increase in frequency this was not observed as regards balance amplitudes The balance excursions, however tended to decrease at all frequencies with a rise in sensation level the differences were significant only between 20 and 80 dB SL at 6000 Hz.

2. ADAPTATION

Perstimulatory suprathreshold adaptation at the four sensation levels and seven frequencies studied during 3 min (Table 3) was characterized by large individual variations, indicated by large standard deviations. In this series there were two subjects (5 per cent) in whom adaptation was almost always at all intensity levels and frequencies below 10 dB only occasionally above 1000 Hz it could exceptionally be higher As contrasted with these two, some of the test subjects regularly showed adaptation of remarkable amount in all frequency SL combinations. Figure 5 presents some individual tracings at 60 dB SL, 4000 Hz. Sensation to an adapting stimulus could disappear completely at 20 and 40 dB SL for the frequencies 2000 3000 4000 and 6000 Hz. This no longer occurred at 60 and 80 dB SL nor was it found at 1000 Hz and lower frequencies at any sensation level.

TABLE 3

Perstimulatory suprathreshold adaptation for continuous pure tones during 3 min
Results on 20- to 45-year old subject

Frequency (Hz)	Sensation level (dB)	Time (sec.) 15 Mean	15 S.D	30 Mean	30 S.D	45 Mean	45 S.D	60 Mean	60 S.D	90 Mean	90 S.D	120 Mean	120 S.D.	150 Mean	150 S.D	180 Mean	180 S.D
250	20	0.6	4.8	1.4	4.8	1.7	5.4	2.7	5.9	2.9	6.5	2.8	6.5	3.3	7.0	3.1	7.2
	40	2.1	7.1	4.5	9.2	5.9	9.2	6.9	9.8	7.6	10.4	8.3	10.6	9.2	10.5	9.3	10.8
	60	2.8	6.7	5.1	8.9	6.2	9.2	7.6	9.5	10.6	9.8	11.2	10.0	12.6	11.5	12.2	11.9
	80	2.7	6.5	4.2	7.9	6.1	8.9	7.3	9.3	9.5	9.6	10.5	9.3	12.0	9.7	11.3	10.1
500	20	0.5	5.3	1.9	6.7	2.5	6.9	3.3	6.6	4.8	7.1	4.6	7.8	5.5	8.2	4.5	7.9
	40	3.4	7.4	5.7	9.4	7.4	10.7	8.2	12.0	9.4	12.6	10.0	12.5	10.6	13.1	10.6	13.2
	60	2.6	6.9	5.5	12.2	8.0	12.6	9.6	13.6	11.5	14.6	13.4	15.4	15.2	15.9	15.2	15.4
	80	4.9	8.4	8.9	13.2	10.8	14.0	11.5	14.3	13.1	13.5	14.9	14.4	16.8	15.0	16.9	15.6
1000	20	0.8	4.8	2.5	5.9	3.9	6.6	5.2	6.8	7.1	6.6	8.0	6.6	9.1	6.5	8.7	7.4
	40	4.5	7.1	7.7	9.5	10.4	11.2	12.0	11.6	14.7	11.2	16.3	12.1	18.1	12.5	18.2	13.3
	60	4.7	8.5	9.5	14.0	12.2	14.9	14.9	15.8	18.6	16.0	21.5	16.1	21.9	15.8	22.6	16.2
	80	4.5	8.4	9.4	14.7	12.5	17.0	14.6	17.9	18.7	19.0	20.6	19.6	22.2	19.5	22.8	19.5
2000	20	3.8	6.4	6.5	8.4	8.7	8.9	9.7	9.2	10.6	9.9	10.8	9.1	11.9	9.0	11.8	8.3
	40	5.5	8.0	11.1	11.0	14.0	12.9	16.2	13.6	18.5	12.8	19.8	12.4	21.4	12.8	21.7	12.3
	60	5.1	9.1	11.0	13.4	15.8	15.7	19.2	17.2	24.3	18.6	26.5	18.4	28.0	18.5	28.3	17.9
	80	5.8	10.4	10.2	13.2	13.6	15.2	16.5	16.1	21.2	18.0	25.4	19.3	27.2	19.9	27.5	19.0
3000	20	4.2	6.2	7.8	7.0	9.8	7.4	11.1	7.8	12.1	8.4	12.9	8.8	13.6	8.1	13.0	8.2
	40	4.2	8.0	8.8	11.0	12.7	11.7	15.0	11.6	17.8	11.7	20.3	11.5	21.1	11.1	20.7	10.2
	60	6.2	8.0	12.8	12.0	17.0	14.2	20.5	15.4	23.9	15.6	27.4	15.1	29.7	15.3	28.6	14.9
	80	4.1	8.7	8.4	12.8	12.1	14.6	15.2	15.8	19.3	18.4	23.3	18.9	26.0	18.7	26.7	20.4
4000	20	3.4	6.4	6.6	7.8	8.6	7.3	9.2	8.0	10.5	8.6	11.2	8.4	11.3	9.0	11.2	8.2
	40	6.9	7.9	11.2	11.2	14.2	11.4	15.8	11.4	17.8	12.0	19.3	11.2	20.3	11.5	20.5	11.2
	60	6.3	8.2	11.9	12.5	16.2	14.6	20.0	15.5	23.6	16.5	27.5	16.8	29.3	16.2	28.4	15.0
	80	4.6	8.9	9.0	13.3	12.1	15.0	14.7	16.1	18.6	15.6	22.6	17.0	25.7	17.4	26.9	18.4
6000	20	3.7	6.0	7.3	6.1	9.2	6.0	10.2	5.6	11.0	5.6	11.3	5.5	11.4	5.3	10.8	5.5
	40	5.2	8.0	10.9	11.1	14.1	11.3	16.7	11.3	18.7	10.3	20.2	10.3	21.9	10.3	21.9	10.4
	60	5.0	7.5	11.0	13.2	15.3	15.9	19.7	16.2	23.5	17.2	26.6	17.3	29.1	17.5	29.2	16.6
	80	5.9	8.8	10.5	12.7	13.4	15.4	15.8	15.8	20.6	17.4	24.3	18.0	26.6	18.5	27.7	17.8

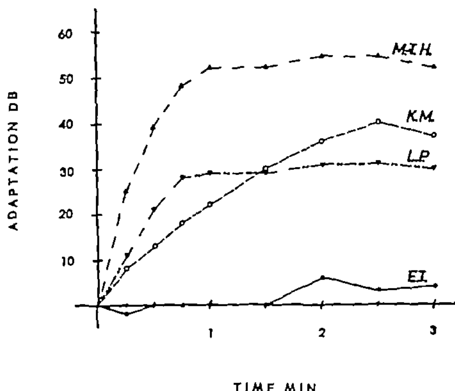
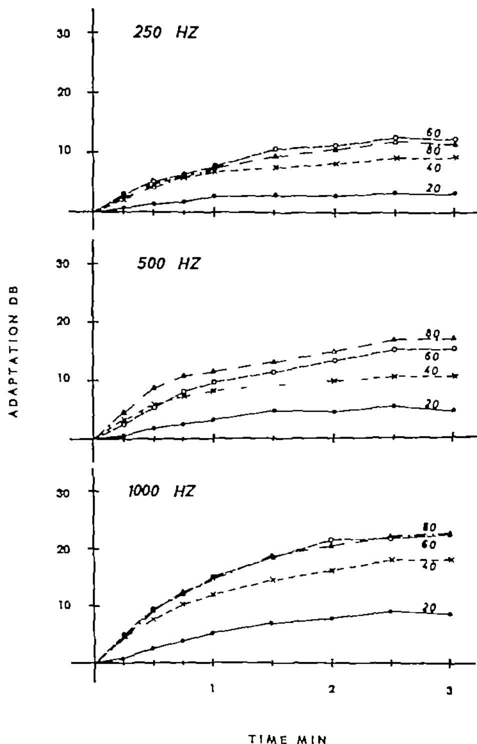


Fig 5 Individual adaptation tracings at 60 dB SL 4000 Hz. Adaptation as recorded for test subject L.P (30 years) and K.M (27 years) was of moderate amount, in the latter the growth of daptation wa slow In test subject M. T H. (22 years) adaptation was large in amount and similar in type to subject L.P The fourth subject here included, E.T (22 years), represents a type with slight daptation t all frequencies and sensation levels.

) ADAPTATION IN RELATION TO SENSATION LEVEL

As illustrated in Figures 6—12, adaptation in the total series behaved in a very regular manner in spite of large dispersions it grew at frequencies 250—6000 Hz with increasing sensation level from 20 dB to 60 dB reaching the values (3 min) 12.2, 15.2 22.6 28.3 28 6 28 4 and 29.2 dB At 250 500 and 1000 Hz the increase was slower at the frequencies 2000, 3000 4000 and 6000 Hz practically linear

When intensity increased from 60 dB SL to 80 dB SL, adaptation did not continue to grow but indeed seemed to diminish at and above 2000 Hz. Yet no significant differences were recorded for any of the frequencies tested at the various points of time Adaptation at 20 dB SL (3 min.) differed significantly for all frequencies from the values measured at 40 dB SL and also at 60 and 80 dB SL However a comparison of the values obtained at 40 dB SL and those obtained at 60 dB revealed significant differences for 2000 3000 4000 and 6000 Hz, but not at 1000 Hz and lower frequencies where the amount of adaptation was less. Between sensation levels of 40 and 80 dB there were no significant differences.



Figs 6—8 Perstimulatory suprathreshold adaptation during 3 min. at 250, 500 and 1000 Hz; sensation level as parameter

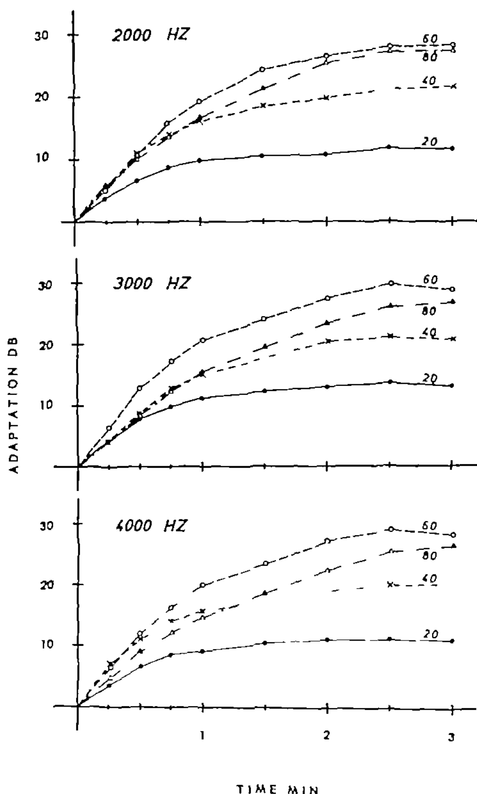


Fig. 9—11. Perstimulatory suprathreshold adaptation during 3 min. at 2000, 3000 and 4000 Hz, sensation level as parameter

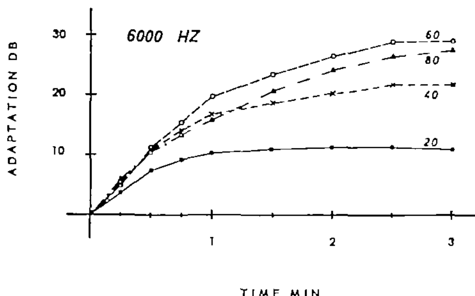


Fig. 12. Perstimulatory suprathreshold adaptation during 3 min. at 6000 Hz sensation level as parameter

b) ADAPTATION IN RELATION TO FREQUENCY

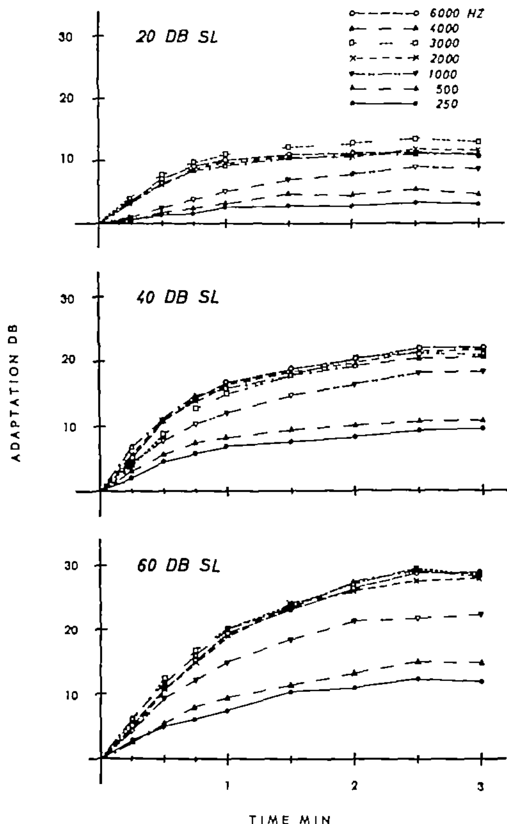
Adaptation for the various frequencies studied at different sensation levels is shown in Figures 13—16 The general conclusion to be drawn is that adaptation is larger at higher than at lower frequencies.

The results at 250 and 500 Hz were along the same lines at each of the four sensation levels studied adaptation seemed to be larger for 500 Hz at all sensation levels but there were no significant differences between these two frequencies at any of the points of time

At the frequencies 2000 3000 4000 and 6000 Hz adaptation was found to be of the same order at all sensation levels under study there were no significant differences at the points of time studied

Adaptation registered for 250 Hz at all four sensation levels was significantly smaller at 3 min than it was for 1000 Hz and higher frequencies. The result at 500 Hz differed from 1000 Hz and higher frequencies at 20 and 40 dB SL the difference becoming significant at 60 and 80 dB SL for 2000 Hz and upwards. The frequency 1000 Hz did not differ from the higher frequencies at any one of the sensation levels.

The amount of adaptation was not affected by the order of frequencies tested, i.e. by whether measurements were started at 60 dB SL from the highest frequency 6000 Hz, proceeding systematically downwards, or from the lowest point, 250 Hz, upwards. The results will be found in Table 4 When testing was started at 250 Hz, adaptation at the low frequencies, most difficult to balance, seemed to be slightly less, the differences, however were not significant for any of the frequencies.



Figs. 13—15 Perstimulatory suprathreshold adaptation during 3 min. at 20, 40 and 60 dB SL; frequency as parameter

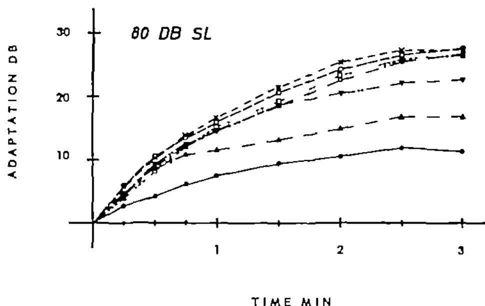


Fig. 16. Perstimulatory suprathreshold adaptation during 3 min. at 80 dB SL frequency as parameter

ADAPTATION IN RELATION TO STIMULUS DURATION

The correlation between the amount of adaptation and stimulus duration is clearly evident in Figures 13—16 As a function of the time required for maximum to be reached, adaptation would seem to be affected by frequency and sensation level alike.

While at 20 and 40 dB SL three fourths of the adaptation occurred during the first minute of the testing run at all frequencies studied, the single exception being 1000 Hz with an apparently slower growth, three fourths of total adaptation was attained as late as after 2 minutes at the sensation levels of 60 and 80 dB Growth continued, however in all frequency SL combinations up to $2^1/_2$—3 min.

As regards the growth rate of adaptation it was possible to separate two distinctly different groups of subjects, but, as already mentioned, there were also a few subjects (5 per cent) in whom adaptation did not appear at all or was generally slight, less than 10 dB

In the other main group of normal subjects, consisting of 19 persons (50 per cent), the growth of adaptation was slow it increased almost in the form of a straight line up to 2—3 min Eight subjects, in 14 different frequency SL combinations, showed sharply increasing adaptation as late as at 3 min. and recording was continued. The growth of adaptation, however did not continue in most of these cases: in only four tests (3 subjects) did adaptation at $3^1/_2$ min exceed the value obtained at 3 min. the testing frequencies were 1000—6000 Hz at 60 and 80 dB SL. The differences compared

TABLE 4

Perstimulatory tone threshold adaptation at 60 dB SL
Descending order of frequency testing compared with ascending order

Frequency (Hz)	Sensation level (dB)	Time (sec.) 15 Mean	S.D	30 Mean	S.D	45 Mean	S.D	60 Mean	S.D	90 Mean	S.D	120 Mean	S.D	150 Mean	S.D	180 Mean	S.D
250	60_I	2.8	6.7	5.1	8.9	6.2	9.2	7.6	9.5	10.6	9.8	11.2	10.0	12.6	11.5	12.2	11.9
	60_{II}	1.9	6.4	5.0	9.8	3.3	10.0	3.8	10.3	4.6	10.2	5.6	10.4	6.2	10.7	6.0	10.6
500	60_I	2.6	6.9	5.5	12.2	8.0	12.6	9.6	13.6	11.5	14.6	13.4	15.4	15.2	15.9	15.2	15.4
	60_{II}	1.5	7.7	3.6	11.5	5.2	11.7	6.7	12.2	9.1	13.5	10.7	14.9	12.5	15.5	12.9	16.5
1000	60_I	4.7	8.5	9.5	14.0	12.2	14.9	14.9	15.8	18.6	16.0	21.5	16.1	21.9	15.8	22.6	16.2
	60_{II}	2.7	7.3	6.0	11.9	8.3	13.3	10.7	15.3	13.7	17.9	16.2	19.9	18.4	19.5	19.0	20.0
2000	60_I	5.1	9.1	11.0	13.4	15.8	15.7	19.2	17.2	24.3	18.6	26.5	18.4	28.0	18.5	28.3	17.9
	60_{II}	4.7	9.2	10.5	14.5	13.2	15.8	16.4	16.6	20.3	16.8	23.2	17.0	24.9	16.5	24.6	16.8
3000	60_I	6.2	8.0	12.8	12.0	17.0	14.2	20.5	15.4	23.9	15.6	27.4	15.1	29.7	15.3	28.6	14.9
	60_{II}	5.0	9.2	10.8	14.9	14.0	16.0	16.8	17.5	20.8	18.5	23.7	17.6	24.3	17.2	25.1	17.7
4000	60_I	6.3	8.2	11.9	12.5	16.2	14.6	20.0	15.5	23.6	16.5	27.5	16.8	29.3	16.2	28.4	15.0
	60_{II}	6.3	9.3	12.6	16.2	16.3	17.7	18.4	18.2	23.5	18.1	27.0	18.4	28.2	18.6	29.7	18.3
6000	60_I	5.0	7.5	11.0	13.2	15.3	15.9	19.7	16.2	23.5	17.2	26.6	17.3	29.1	17.5	29.2	16.6
	60_{II}	6.7	8.9	13.8	15.6	17.1	16.5	19.4	17.9	22.7	18.5	26.3	18.0	29.3	18.0	29.2	18.4

60_I = Frequencies tested from 6000 Hz downward
60_{II} = Frequencies tested from 250 Hz upwards

with the 3 min. values were 3 dB in 3 cases, 5 dB in one. In one subject an increase still appeared at 3½ min. but the adaptation value for 4½ min. equalled the one for 3½ min.

In 17 subjects (45 per cent) adaptation developed rapidly at most of the frequencies and sensation levels and was complete within the first minute. Two test subjects stated that the audiometer rate of 2.1 dB/sec. failed to keep up with adaptation at 40–80 dB SL for any of the frequencies during the first few seconds. One of them said the stimulus remained unchanged for a few seconds, then decreased very strongly for a few more seconds, after which it was again stabilized for up to 3 minutes. The adaptation value at 15 sec. was recorded as the audiometric value, though it was evidently greater

d) CHANGES IN PURITY OF THE ADAPTING STIMULUS

Adaptation was associated with another subjectively recognizable feature besides the decline of loudness, i.e. the continuous pure tone stimulus changed in quality becoming less pure There was not a single test subject who did not report this finding at some one of the frequencies tested, taking all sensation levels into consideration if adaptation was demonstrable The majority stated of their own accord that, irrespective of the frequency the adapting stimulus began to resemble a noise or was either duller or in some way lower than the comparison tone. Three out of 38 test subjects described the stimulus as being cracked. Table 5 shows the results in terms of number

TABLE 5

Changes in purity of adapting stimuli

Frequency (Hz)		Sensation level (dB) 20 N	%	40 N	%	60 N	%	80 N	%
250	Changed	0		0		1	3	0	
	Pure	32	100	32	100	31	97	27	100
500	Changed	2	6	5	16	4	12	6	19
	Pure	30	94	27	84	28	88	26	81
1000	Changed	6	19	11	34	11	34	10	3
	Pure	26	81	21	66	21	66	22	68
2000	Changed	18	56	20	62	19	59	18	56
	Pure	14	44	12	38	13	41	14	44
3000	Changed	22	69	25	78	21	66	21	66
	Pure	10	31	7	22	11	34	11	34
4000	Changed	27	84	25	78	28	88	27	84
	Pure	5	16	7	22	4	12	5	16
6000	Changed	25	78	26	81	26	81	27	84
	Pure	7	22	6	19	6	19	5	16

N = Number of stimuli

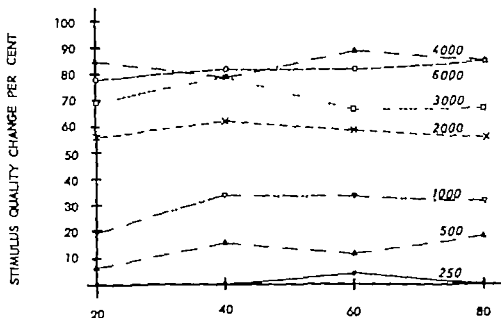


Fig 17 Changes in quality of adapting pure tone stimuli; number of cases with changed quality expressed as percentage

of changed and pure stimuli The results are illustrated in Figure 17 with frequency as parameter Tested at individual frequencies, the quality changes did not differ in principle from one sensation level to another in the series as a whole. But it was found that with rising frequency the proportion of cases with changes in purity increased definitely up to 4000 Hz, equalling, however at 6000 Hz the figure for 4000 Hz at all sensation levels. Between 1000 and 2000 Hz the number of cases with changes in tone quality became greater than the number of those remaining unchanged in purity

It was the rule that, in those cases in which the sensation to the adapting stimulus disappeared it first lost its purity Even though there was a rough correlation between changes in purity and decline in loudness level, no distinct regularity was found in individual cases. The change was in some cases connected with loudness decline of very marked degree and in others with slight loss of loudness. On the other hand, in cases in which the decline in loudness was of more than average amount, the stimulus occasionally remained pure even at the highest frequencies.

Some of those tested were able to define the moment at which the change occurred, usually within the first minute however in a few cases it did not appear until at the later stages of the test, regardless of the degree of loudness decline. There were also some who could not define the exact moment, stating that the change was gradual.

) EXCURSION WIDTHS OF ADAPTATION TRACINGS

Table 6 lists the mean amplitudes of the adaptation curves and the standard deviations, during the first minute and for the fully adapted ear The minimum and maximum values were 2 and 16 dB at the beginning of the test run and 2 and 17 dB respectively at its end most of the values being within the range 5—10 dB In Figure 18 are seen

TABLE 6

Excursion widths (dB) f adaptation tracings

Frequency (Hz)	Sensation level (dB) 20				40				60				3			
	Beg.		End		Beg.		End		Beg.		End		Beg.		E d	
	Mean	S.D	Mean	S.D	Mean	S.D	Mea	S.D.	Mean	S.D	Mean	S.D	Mean	S.D	\	S.D
250	6.9	2.9	7.2	3.0	7 1	2.5	7.0	2.8	6.8	2.6	6.8	2.7	6		6 6	3
500	7.2	2.6	6.8	2.6	7.2	2.6	7 1	2.6	6 6	2.2	6.6	2.4	6 7	8		3
1000	6.9	2.5	6.5	2.8	7.5	2.6	7.0	2.6	7.0	2.1	7 1	2.6	6.7	2 8	6	3
2000	6.8	2.5	6.5	2.6	7 1	2.3	7 1	3.2	6.7	1 9	7.0	2.6	7..	2 8	6	1
3000	7 4	2.8	6.7	2.9	7 4	2.3	7 4	2.9	6.9	2.1	7.2	2.6	6.9	2 4	7	3
4000	7.2	2.4	6.6	2.5	6.8	2.3	6.8	2.4	6.8	2.4	6.8	8	6.4	2.6	6 6	2 9
6000	7 9	3.2	7.0	3 1	6.9	2.1	6.5	2.1	6.6	1 9	6.8	2.8	6.3	2 3	6 5	2

Beg. = Developing adaptation
End = Adaptation fully developed

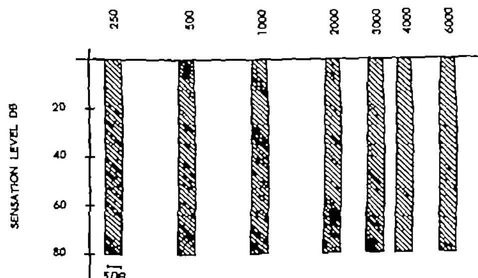


Fig 18 Excursion widths of adaptation tracings in the fully adapted compared with threshold amplitudes f the control

the averages for the fully adapted ear with threshold amplitudes of the control ear (Table 2 page 29) As regards the values for developing and for fully developed adaptation no significant differences were observed between any of the SL frequency combinations in the various groups nor between the groups. Neither were differences found when the values were compared with the corresponding prestimulatory balance amplitudes (Table 2 page 29) The initial values behaved in the same way as the latter group in the respect that they also showed a tendency to decrease with increasing sensation level. It was not possible to make the same observation at the end of the tracing

3 POSTSTIMULATORY THRESHOLD SHIFT

Table 7 analyses the mean poststimulatory threshold shift, with standard deviations measured with an interrupted tone 60 sec. after cessation of the stimulus. For an overall picture of recovery those cases in which stimulus duration exceeded 3 min (12 recordings 3½ min one 4 min., one 4½

TABLE 7

Poststimulatory threshold shift 1 min after cessation of adapting stimulus

Frequency (Hz)	Sensation level of stimulus (dB) 20 Mean	20 S.D	40 Mean	40 S.D	60 Mean	60 S.D	80 Mean	80 S.D
250	2.7	2.7	3.0	3.5	4.2	4.9	2.9	2.9
500	3.0	3.0	2.7	2.9	2.8	3.3	2.6	3.1
1000	3.2	3.2	3.2	2.7	4.0	2.6	3.2	2.7
2000	4.1	3.0	3.6	3.4	4.0	3.2	3.3	2.1
3000	3.2	2.9	2.9	2.8	3.8	2.9	2.8	2.9
4000	3.2	2.9	3.6	3.2	3.3	2.4	3.6	2.6
6000	4.3	4.6	3.7	4.1	4.7	3.6	3.9	3.5

TABLE 8

Excursion widths (dB) of poststimulatory threshold tracing compared with immediate prestimulatory threshold amplitudes

Frequency (Hz)	20 Pre Mean	20 Pre S.D	20 Post. Mean	20 Post. S.D	40 Pre. Mean	40 Pre. S.D	40 Post. Mean	40 Post. S.D	60 Pre. Mean	60 Pre. S.D	60 Post Mean	60 Post S.D	80 Pre Mean	80 Pre S.D	80 Post. Mean	80 Post. S.D
250	6.2	1.8	5.8	1.9	6.5	1.7	6.0	1.8	6.3	1.9	5.8	1.8	6.3	2.0	5.9	1.9
500	6.2	2.2	5.5	1.8	6.2	1.8	5.9	1.8	6.0	1.9	6.0	[illegible]	6.1	1.8	5.9	2.1
1000	5.7	2.0	5.5	2.0	5.4	1.5	5.8	1.8	5.7	1.6	5.8	2.0	5.6	1.6	5.8	1.9
2000	6.0	1.8	5.4	1.8	5.9	1.7	6.8	1.8	5.4	1.9	5.4	2.0	5.8	1.7	5.5	1.7
3000	5.6	1.8	5.7	1.6	5.8	1.4	5.6	1.9	5.5	1.6	5.7	2.0	5.5	1.5	5.9	2.0
4000	5.8	1.7	5.6	1.8	5.6	1.6	5.4	1.6	5.1	1.3	5.4	1.9	5.2	1.6	5.4	1.7
6000	5.6	1.5	5.4	1.6	5.4	1.8	5.2	1.6	5.0	1.4	5.3	1.6	5.2	1.4	5.5	1.5

Pre. = Prestimulatory threshold
Post. = Poststimulatory threshold

min.) were also included. There was a tendency for threshold loss to increase with increasing frequency but the difference was not significant at any of the sensation levels. It is worth noticing that the results at the various sensation levels also did not differ statistically for any one of the frequencies tested.

The excursion widths of the prestimulatory (the ear under test) and poststimulatory threshold tracings at the end of the first minute are given in Table 8. The means show no significant differences for any frequencies. Similarly as the prestimulatory values, the poststimulatory values tended to decrease as the frequency increased.

Sixteen test subjects (42 per cent) reporting that the adapting stimulus lost its pure quality also stated that the poststimulatory threshold tone was impaired. This finding usually referred to the early phase of recording and almost exclusively to frequencies above 1000 Hz, and at these frequencies irregularly to all sensation levels. In this part of the study observations are necessarily subjective and uncertain because there were no possibilities of comparison.

B. CHILDREN AND OLD AGE GROUP

Children

It appeared in the preliminary tests that practically no adaptation was demonstrable in subjects below 15 years of age though adaptation did occur in most of the subjects above this age limit. On the basis of experience gained in the normal group and in the preliminary tests, and with a view to ease of comparison and the greatest possible amount of adaptation, this part of the study was limited to testing 29 subjects aged 7–15 years at 60 dB SL, twenty of these at 2000 Hz and 23 at 4000 Hz. The results are shown in Table 9. Adaptation in excess of 10 dB (11–28 dB) in 3 min. was found in only 5 subjects. These were

TABLE 9

Perstimulatory suprathreshold adaptation at 60 dB SL in 7 to 15 year old subjects

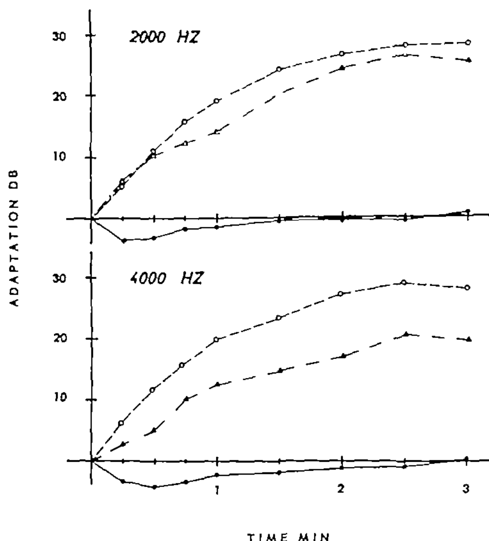
Time (sec.)	Frequency (Hz) 2000		4000		Remarks
	Mean	S.D	Mean	S.D	
15	—3.6	3.8	—3.3	4.6	Average age 12 y
30	—3.2	4.3	—4.3	5.4	Number of subjects
45	—1.6	4.8	—3.6	6.2	20 at 2000 Hz and
60	—1.2	5.5	—[illegible].4	6.8	23 at 4000 Hz
90	—0.2	5.9	—2.1	7.2	
120	—0.2	6.9	—1.[illegible]	8.1	
150	—0.3	7.5	—0.9	8.7	
180	1.0	8.4	0.3	10.6	

TABLE 10

Excursion widths (dB) of prestim latory balance tracings and of adaptation tracings at 60 dB SL compared with threshold amplitudes / control ear (same subjects as in Table 9)

Frequency (Hz)	Thr Mean	Thr S.D	BaL Mean	BaL S.D	Beg. Mean	Beg. S.D	End Mean	End S.D
2000	7.2	2.2	7.4	2.1	8.0	2.8	7.8	2.8
4000	7.1	2.0	8.0	2.1	7.2	2.4	7.6	3.1

Thr = Threshold
BaL = Prestimulatory balance
Beg = Developing adaptation
End = Adaptation fully developed



Figs 19—20 Perstimul tory suprathreshold daptation h ldren (———) and m the old ge group (— —) measured 60 dB SL fo 2000 a d 4000 Hz compared w th the ormal group consi ti g f 20—45-) ld subjects (o— — —o).

without exception among the oldest in their group (12 to 15 years) The results together with the tracings obtained for the normal group and for old people are found in Figures 19 and 20 The differences when compared with the normal group are significant for the two frequencies at each point of time The negative values are accounted for by the automatic attenuation mechanism of the audiometer with the switch in the resting position the intensity increases and absence of response for 1–2 sec. at the beginning of stimulation means a decline of 2.1–4.2 dB

The prestimulatory balance levels at 2000 and 4000 Hz were 61 6 dB S.D 11.8 and 63.5 dB S.D 9.7 respectively the latter figure exceeds the one obtained for the normal group (P < 0 01) Table 10 compares the threshold amplitudes with those of prestimulatory balance tracings and of adaptation tracings. Apart from the threshold amplitudes, the values did not differ statistically from those of the normal group and their interrelationship was the same as in the normal group with the one exception that there was no significant difference between threshold amplitudes and prestimulatory balance amplitudes. In respect of poststimulatory threshold loss, the children did not differ from the normal group (2000 Hz, 4 6 dB S.D 4.2 4000 Hz, 4.6 dB S.D 5 4) As in the normal group there was no difference between poststimulatory and prestimulatory threshold amplitudes in the children s group

Old age group

Table 11 lists the results of adaptation measurements at 60 dB SL in 10 subjects aged 56 to 71 years. The values for 2000 and 4000 Hz are compared in Figures 19–20 with the corresponding results in the normal group and the children s group At and above 3000 Hz, where the age-linked threshold loss appears most clearly adaptation seemed to diminish in amount with increasing threshold loss. The difference compared with the normal group however was not of significance until at 6000 Hz (P < 0 005)

Two subjects had so much elevated thresholds that the test at 6000 Hz could not be made at 60 dB SL and was possible at the level of 40 dB In each of these cases the recorded result was lower than the means in the normal group

TABLE 11

Perstimulatory suprathreshold adaptation at 60 dB SL in 10 old (56—70 y) subjects.

Frequency (Hz)	15 Mean	15 S.D	30 Mean	30 S.D	45 Mean	45 S.D	60 Mean	60 S.D	90 Mean	90 S.D	120 Mean	120 S.D	150 Mean	150 S.D.	180 Mean	180 S.D
250	1.2	5.3	4.0	7.0	4.2	7.9	3.7	8.9	6.9	8.3	8.4	8.3	9.6	8.8	10.7	8 9
500	1.1	5.8	2.8	5.3	2.9	5.3	3.2	6.0	5.3	7 4	9 4	9.6	11 9	10.4	12.9	12.7
1000	0.9	6.3	4.5	10.1	7.0	11.6	8.5	12.7	11.7	14 4	13.9	14.5	16.3	13.6	18.7	15.0
2000	5 9	6 9	10.2	7 9	12.2	9.7	14 1	13.8	20.2	16.6	24 4	15 1	26.6	16.9	25.3	16.1
3000	2.3	7 8	7.3	8.3	11.0	7.9	13.3	8 4	16.4	8.8	19 9	10.4	22.5	9.0	22.8	10.2
4000	2.7	4 0	5.0	6.1	10.1	10.6	12.6	15.2	14.8	19.3	17 1	18 4	20.8	18.6	19 9	17.2
6000	2.2	4 6	4.6	5.8	7.0	6.2	10.0	8.2	12.2	8.5	16.2	9.7	17.0	11 4	17 9	10.8

intensities, adaptation occurred in some ears to the extent that the sensation to the continuous tone disappeared nevertheless, the recognition of the additional impulses was not different from that found in other ears in the same group

A 200 msec. pulse is also sufficient for the threshold minimum as well as for maximum loudness at the suprathreshold levels to be obtained not only in the normal ear (Miskolczy Fodor 1959 Small et al. 1962) but also in hearing impairments of different types (Miskolczy Fodor 1953) According to Jerger and Jerger (1966) a 200 msec interval at threshold level suffices for recovery of a functional change caused by a 200 msec. pulse in such lesions also in which perstimulatory threshold adaptation is of large amount.

Prestimulatory balance

When the audiometer channels have identical basic levels (re 0.0002 dyn/cm² SPL) the balance level reached in the control ear should be equal, in decibels, to the sensation level of the normal test ear if adaptation or other functional changes occur in neither ear The results indicated (Table 1 page 29) that, with increased sensation level the balance level tended to be reduced in relation to it. There are no previous data on this phenomenon but the same tendency is apparent from Egan's (1955 a) study and indeed is of the same order of magnitude (2—5 dB) at the corresponding sensation levels, as in the present study

The result is probably explained by binaural interaction this in fact has been found to modify nearly all auditory functions. At high intensities, in addition the part played by the stapedius reflex cannot be excluded if stimuli are used which exceed the latency period of the reflex in duration.

Prestimulatory balance might also be performed using alternate impulses. Changes in interaural relations then could not fail to affect the balance levels to some extent. Dirks and Malmquist (1965) for instance, have obtained auditory threshold shifts from 1 to 4 dB according to whether the contralateral suprathreshold 0 4 sec. stimulus was presented simultaneously or alternately with the test tone.

Adaptation

According to amount and growth rate of adaptation the test subjects can be divided into three groups the typical tracings appear also in Figure 5 (page 32)

— Type I, represented by test subject E. T., is characterized by total absence or generally minimal amount of adaptation (less than 10 dB) This type included 2 subjects (5 per cent) of the normal group and this seems to be the predominant type in the case of children

— In type II including subject K. M. the growth of adaptation is slow but may reach a large value Nineteen subjects (50 per cent) in the normal group were representative of this type.

— Type III (test subjects L. P and M. T H) is characteristically associated with rapid decline in loudness level and there are then no significant changes after 60 sec. Seventeen (45 per cent) of the adults tested were of this type.

One and the same subject shows the same type of tracing at most frequencies and sensation levels. Using 250 500 and 1000 Hz stimuli however a tendency was observed in some cases for type III to shift analogously to type II and for type II to shift to type I This was also noted in the same test subjects above 2000 Hz at 80 dB sensation level Despite large individual differences, perstimulatory suprathreshold adaptation in one and the same subject is a very stable phenomenon — as demonstrated also by Harbert et al. (1966).

Considering the series as a whole, adaptation increased with sensation level from 20 dB up to 60 dB yet the value for 80 dB SL was essentially the same at all frequencies as for 60 dB (Figs. 6–12, pages 33–35) The same conclusion was formed by Palva (1955), Wright (1959) and Tsuiki (1965) It is clear beyond doubt that some additional factor becomes operative at high intensity levels. According to the theory advanced by Keidel (1966) the inner hair cells, having a threshold about 60 dB higher than the outer hair cells, are adapted to a less extent than the latter which might signify a slower growth of adaptation from 60 dB SL upwards, and so explain the result.

As a function of frequency the amount of adaptation, expressed in decibels, appears to behave similarly irrespective of the testing method used Growth was roughly linear with the frequency up to 2000 Hz at all sensation level and then remained practically unchanged at 3000 4000 and 6000 Hz (Figs 13–16 pages 36–37). As a function of both sensation level and frequency types II and III behaved in the same way

In type III however the individual values were inclined to be higher than the means for the total series in all frequency SL combinations in type II by contrast, slightly lower There were no inter-group differences as regards the maximum adaptation values for example at 4000 Hz and 60 dB SL, it was in type II 51 dB and in type III 53 dB

In respect of the time factor types I and III are easily explained: in the former there was no adaptation whatever in the latter no significant changes occurred after 60 sec. In type II on the other hand, there was growth of adaptation up to 2 1/2–3 min in all frequency SL combinations, from which it follows that adaptation also increased in the total series for as long as 2 1/2–3 min Measurements were made on 8 subjects for over 3 minutes, and in three of these (a total of 4 tests) adaptation prowed to be of greater amount at 3 1/2 min In three frequency SL combinations the increase was 3 dB and in one 5 dB.

In general terms, adaptation reaches a maximum in 3 minutes but growth may continue beyond this time limit in a number of normal-hearing subjects tested with 1000 Hz tones or higher at 60 and 80 dB SL.

Egan (1955 a), Thwing (1955), Egan and Thwing (1955), Jerger (1957) and Wright (1959) attained maximum adaptation at 6–7 min. using a

balance with a 15 sec. continuous tone With 10 sec. duration of balance, Hood (1950) reached the maximum in 3–3½ min In all the above studies the growth of adaptation proved slower than it was with the present short comparison tone This is due to the adaptation occurring in the control ear since in the early part of the test, when adaptation in the ear being tested was still slight, the error caused by adaptation in the control ear is relatively larger than at a later stage, from which it follows that balance is also reached later

Using 200 msec comparison signals the degree of adaptation in 15 sec. in type III was, for the frequencies 1000–6000 Hz, 10–20 dB at the sensation level of 20 dB and 10–30 dB at the levels 40–80 dB, thus a considerable error arises from the use of a continuous test tone when the duration of balance is 15 sec. In type II with slowly developing adaptation however it is possible to obtain relatively true values and measure large amounts of adaptation by this technique too

A comparison of the mean values here obtained with earlier studies shows that they do not greatly differ from the corresponding values reported by Egan (1955 a) Thwing (1955) Jerger (1957) Wright (1959) and Small and Minifie (1962) This is understandable seeing that, in types I and II — to which more than half (55 per cent) of the test subjects belonged — the error due to a long balance time is comparatively small. In the total material here studied, adaptation at 15 sec. was on average 4–6 dB at all sensation levels measured for the frequencies from 1000 to 6000 Hz. (Table 3 page 31) In type III there was the greatest source of error but as adaptation is nearly always large, the error as far as means are concerned does not reach a remarkable amount in the series taken as a whole. If however comparisons are made at 3 min (Palva 1955 Jerger 1957 Wright 1960 Small 1964) the values of the investigators mentioned in the preceding are 5–10 dB lower in the range 2000–6000 Hz at all sensation levels. By contrast, below 1000 Hz there are no appreciable differences because adaptation here is of less amount and the maximum appears earlier as pointed out by Jerger (1957) In Hood's (1950) study alone, based on very few test subjects, the values at 60 and 80 dB SL (at 1000 Hz) were about 10 dB higher than those given by others. Wright (1960) who used 1 sec comparison stimuli, obtained for 500 Hz at 90 SPL some individual adaptation values of 50 dB and Palva (1964) has reported comparable results with the same technique.

The use of a short-duration comparison stimulus in a proportion of cases (adaptation type III) results in decisively higher amounts of adaptation than is obtainable with a long continuous control tone. The error occasioned by adaptation in the control ear does not, however alone explain the differences between the results of various investigators (the balance time, for instance, being equal) The different results must be basically due to chance differences in the composition of the series, consisting as they do of different adaptation types Even the studies most often quoted (Hood

1950 Palva 1955 Egan 1955 a, Thwing 1955 Jerger 1957 Wright 1959) are based on averages for a small number of subjects, almost without exception less than 10 and this being the case, the results have very likely been affected by such factors as outlined above

It should be noted that no attention has been paid in previous studies to the association between the age of subjects and auditory adaptation. The difference in adaptation between the children and the adults is of great interest, and it is not here a question of possible unreliability of results obtained with an automatic audiometer in children. Stark (1965 1966) carried out threshold measurements with a Békésy audiometer on 5—11 year old children using both interrupted and continuous stimuli, and he demonstrated that from the age of 8 years the results were as stable as for adults. Delany et al. (1966) correspondingly arrived at 6 years of age they used an automatic Rudmose audiometer The results in the present study too, can be regarded as representative. Children and adults showed consistent results in every respect except that the former had a higher prestimulatory balance level at 4000 Hz and larger threshold amplitudes, which therefore did not differ from the prestimulatory balance amplitudes, as they did in the adult normal group The differences in favour of the normal group may be explained in part by the experience of subjects with the recording technique resulting from more numerous test occasions — a fact which Palva (1957) has drawn attention to The relations between the excursion amplitudes were otherwise similar to those in the normal group The poststimulatory threshold curve also proved identical in type with the curve for adults and shows that a child s ear does not adapt to an interrupted test tone Nor was any one of those tested who showed no adaptation able to report a subjective loudness decline of any kind or any change in tone purity which features were typical in the adult group and a criterion for adaptation.

One possib lity of error in the case of children is the development of a strong subjective absolute intensity constant, referred to by Egan (1955 a) With the object of throwing light on this question 10 subjects were tested for adaptation (4000 Hz) so that the measurements were started at 40 dB SL, intens ty being increased unexpectedly by 20 dB after 1¹/₂ min The balance level increased from 41 1 dB S.D 12.6 (1¹/₂ min) to 57.3 dB S.D 10 1 (2 min.) The prestimulatory balance level had been 41 9 dB S.D 10.3 and at 3 minutes — 1 /₂ minutes after the ntensity increase — balance was 56 9 dB S.D 9.5 The obtained adaptation values d d not differ significantly from the corresponding prestimulatory balance levels. None of the test subjects failed to notice the increase in intensity Thus no adaptation was *found* this factor then is not a decisive one.

Adaptation may be modified by central fact rs (Hahn and de M chelis 1960, Wernick and Tobias 1963 Capps and Collin 1965 Collins and Capps 1965) and t may be modified by contralateral st mulation (Hahn d de Michelis 1960 Brunetti 1960, 1961 Hahn 1962 1963 Marpetiol et al 1962) E en though the attitude of ch ldren to the test d ffered from that of ad lt this factor

balance with a 15 sec. continuous tone. With 10 sec. duration of balance, Hood (1950) reached the maximum in 3—3½ min. In all the above studies the growth of adaptation proved slower than it was with the present short comparison tone. This is due to the adaptation occurring in the control ear since in the early part of the test, when adaptation in the ear being tested was still slight, the error caused by adaptation in the control ear is relatively larger than at a later stage, from which it follows that balance is also reached later

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made systematically from the lowest frequency upwards or vice versa Using the former order however results at the lowest frequencies seemed to be slightly lower (Table 4 page 38) If a fatigue effect or a cumulative adaptation played a part, these should be present at the highest frequencies. The fatigue phenomenon in particular has been found to extend above the stimulus frequency and 4000 Hz, from the point of view of fatigue, is both the most effective stimulus and the one that is most sensitively modified (Davis et al. 1950) in addition, adaptation is maximal at frequencies exceeding 2000 Hz.

In the light of previous studies the recovery of adaptation as measured by the technique of Hood, occurred in 1 min. Carterette (1955) using a 30–87 dB SPL noise stimulus (white noise 100–5000 Hz) reported a recovery period of 4 min. Egan (1955 a) and Wright (1959) applied a 7 min. pure tone stimulus (250–4000 Hz) at 90 dB SPL and at 3 min measured 3 dB residual adaptation using a continuous balancing tone beyond this point recovery was not followed.

Changes in purity of the adapting stimulus

On inquiring about the test subjects own observations — something that Simmons and Dixon (1964) consider very important in all audiometric tests — it appeared that adaptation had another subjective characteristic besides loudness level decline namely a change in quality of the pure tone stimulus. It appeared to sound lower than the comparison tone, as described also by de Maré (1939) or the stimulus began to resemble noise. The ability of the ear to detect changes in quality is a very subjective characteristic which seems to account for the fact that there was no definite correlation between loudness level loss and changes in purity in the individual cases. *But if there was* no decline in loudness level, the subjects did not show any change in stimulus purity either In the normal group however the change in quality behaved in much the same way as loudness decline as far as frequency was concerned the frequencies 250 and 500 Hz were in a group of their own whereas the results at 2000–6000 Hz were relatively similar as compared with the others (Fig 17 page 40) When passing from 1000 Hz to 2000 Hz, the proportion of distorted stimuli became greater as compared with those remaining unchanged. It aroused interest that at the individual frequencies the change in quality did not differ in principle from one sensation level to another This is analogous with loudness level decline in that the loudness loss at 20–60 dB SL always represents the same proportion of the stimulation level at each individual frequency It is of slighter amount at the level of 80 dB only Thus the quality change at each sensation level is related to a functional change of definite degree.

Excursion widths of prestimulatory balance and adaptation tracings

The threshold amplitudes (Table 2, page 29) are of the same order of magnitude as the figures reported by numerous experimenters using the same

technique Amplitude showed a tendency to diminish with a rise in frequency — a trend apparent also from the studies of Palva (1956 a 1957)

Prestimulatory balance amplitudes tended to exceed the threshold values in all age groups. The differences were not significant in the normal group between 250 and 1000 Hz, for which the highest threshold amplitudes seemed to be recorded, nor were they significant in the children s group The tendency to decrease with increasing frequency as observed in the case of threshold amplitudes, did not appear in prestimulatory balance amplitudes. With rising sensation level, however there was a tendency for prestimulatory balance amplitudes to be reduced at all frequencies. An explanation is perhaps that the intensity difference limen as is well known decreases as sensation level increases and cannot fail to affect the balance amplitudes

Diminution of amplitude with increasing sensation levels appears also in the adaptation values at the early stage which latter do not differ from prestimulatory balance amplitudes in any respect. This diminution is probably based on the fact that the change in auditory function as regards prestimulatory balance is still slight. Blegvad and Terkildsen (1966) and Blegvad (1967) studying the effect of continuous stimulation of one ear on the threshold amplitudes of the contralateral ear obtained significant differences at 1000 and 4000 Hz with white noise of 50 and 70 dB SPL, using both continuous and pulsed test tones This interaural effect did not appear in suprathreshold balances.

The calculation of adaptation values for the early part of the balancing period was associated with difficulties in type III adaptation. In a number of cases the excursion widths could be determined immediately on starting stimulation for 15—20 sec If adaptation was of large amount from the first few seconds on, then the tracing was sharply rising and excursion amplitudes could not be recorded until the rise began to be stabilized, i.e when adaptation had already largely occurred That is why it was necessary to calculate the values for beginning adaptation having regard to the entire first minute and during a comparatively short time.

The values for the latter part of balancing did not present the same difficulties these were determined for the period immediately preceding the cessation of the adapting stimulus. Statistically they did not differ significantly from the corresponding initial adaptation values nor did they differ from prestimulatory balance amplitudes. This shows that the sensibility of the ear in the adapted state, expressed with the aid of the control ear is not significantly altered Luscher and Laepple (1958) who used the modulation technique to study the intensity difference limen at 80 dB SL, also failed to reach any results diverging from the normal However in the fully adapted ear it was not possible to demonstrate the tendency for amplitudes to decrease with increasing sensation level which appeared in the pre stimulatory balance amplitudes.

Poststimulatory threshold

The decline in poststimulatory threshold 60 sec after cessation of the adapting stimulus at all frequencies and sensation levels varied within 2.6 dB S.D 3 1 (500 Hz, 80 dB SL) and 4.7 dB S.D 3 6 (6000 Hz, 60 dB SL) The differences, compared with prestimulatory threshold, are significant. The values are of the same order as those reported by Hirsh and Bilger (1955) who used the same measuring technique and 2—4 min. stimulation of equal intensity Introduction of a contralateral pulsed signal during stimulation seems not to affect the poststimulatory threshold shift. Neither did Hirsh (1957 1958) find any differences between binaural and monaural stimuli in this respect. Experimenters in general have measured poststimulatory threshold shift with a continuous test tone the result thus being modified by the adaptation caused by the test tone itself and the resulting threshold values have therefore been higher (Hood 1950 Epstein and Schubert 1957 Palva 1958)

It is noteworthy that the sensation level in the range 20—80 dB had no effect on the result, a fact stressed also by Reger and Lierle (1954) when describing their results after 1 min. stimulation at 1000 Hz. At intensities above a fatigue limit, however the correlation with threshold shift proved definite Thus may be taken to indicate that the receptors under physiological conditions always respond to a continuous stimulus in much the same way and recovery after the exposure occurs similarly when measured at 60 sec

In children and in those adults for whom no suprathreshold adaptation was demonstrated, poststimulatory threshold shift was nevertheless equal to that found in adults with a large amount of adaptation This is in favour of the fact that perstimulatory suprathreshold adaptation and poststimulatory threshold shift are physiological processes of differing types.

All organ functions, hearing for instance are associated with katabolic and anabolic processes of the metabolism stimulation has a definite upper limit above which the organism loses itse power of compensation, and fatigue in the proper sense of the word ensues. In regard to hearing this can be demonstrated by psychoacoustic (Hood 1950, Rüedi 1954 Jerger 1956 1958 Epstein and Schubert 1957) and electrophysiologic (Gisselsson and Sorensen 1959 Burgeat and Burgeat Menguy 1964) measurements, but also in the form of histochemical changes in the sensory cells (Vosteen 1958 1961) and electron-microscopic changes in the mitochondria (Spoendlin 1958 Koide et al. 1960). According to Shimizu and his co-workers (1967) fatigue and adaptation were both associated with a decrease in electrical impedance between endolymph and perilymph which seemed to reflect changes occurring on hair cell membranes Very likely the threshold shift following the physiological stimulus is an expression of katabolic processes like those referred to above and related to acoustic stimulation This also seems to be the case, at least partly with adaptation at threshold level which for 22 children included in this study

tested at 4000 Hz, and for 17 tested at 2000 Hz, was of the same magnitude at 3 min (6.7 dB S D 7 9 and 4.3 dB S.D 5.7 respectively) as in a normal hearing adult, measured with a Békésy audiometer continuous stimulus (Palva and Palva 1961) Perstimulatory suprathreshold adaptation on the other hand, might represent a regulation mechanism, mainly by means of the efferent bundles, of the response to a continuous acoustic stimulus, and so it might, in man, be partly the result of a development connected with advancing age.

Poststimulatory functional changes did not appear in the form of changes in threshold amplitudes. The poststimulatory values were not different from the corresponding prestimulatory readings at any of the frequencies studied. The same conclusion was reached by Silbiger and Elliot (1962) under comparable experimental conditions.

VII SUMMARY

The aim of the present study was to measure perstimulatory suprathreshold adaptation, using the decline in loudness level of a pure tone as criterion The testing technique consisted of continuous binaural balancing of the stimulus with an interrupted comparison tone of equal frequency the pulse and pulse-intervals being of 200 msec. duration. With this pulse an onset response is obtained also in the adapted ear

The basic tests were made on 38 normal hearing subjects aged 20—45 years (average 25.4 years) using tones of 250 500 1000 2000 3000 4000 and 6000 Hz at sensation levels of 20 40, 60 and 80 dB in such a manner that each level was tested for 32 different subjects. Adaptation was recorded as decibel units of the balance level determined with interrupted stimuli.

The prestimulatory balance level showed a tendency to decrease in relation to sensation level the means for the various frequencies at 20 dB SL varied from 19.5 to 21.8 dB at 80 dB SL from 75.2 to 80.0 dB (Table 1 page 29) Furthermore, the balance level tended to be lower at the lowest frequency 250 Hz, and at the highest frequencies (3000—6000 Hz) than it was for the middle frequencies.

Adaptation is a very stable phenomenon in one and the same subject, but individual differences proved very large. On the basis of this latter fact and the rate of growth, three types of adaptation emerged

— Type I There is no adaptation whatever or it is very slight (less than 10 dB) in most frequency SL combinations. This type included 5 per cent of all subjects.

— Type II Adaptation develops slowly generally reaching a maximum in 2½—3 minutes exceptionally at 60 and 80 dB SL, later Adaptation may be of very large amount. This type was represented by 50 per cent of the subjects tested.

— Type III: This is characterized by rapid growth of adaptation. After 60 sec., adaptation does not change significantly and it tends to be on average of greater amount than in type II This type included 45 per cent of all subjects.

The adaptation obtained, in the total series, during 3 min. for the seven frequencies studied and the four sensation levels is shown in Table 3 (page 31). As a function of frequency adaptation increased from 250 Hz to 2000 Hz but then remained unchanged up to 6000 Hz, at all sensation levels. Whereas 250 Hz did not differ from 500 Hz, adaptation for these two frequencies was significantly less than that recorded for 1000 Hz and higher frequencies at all sensation levels, except for 500 Hz v 1000 Hz at 60 and 80 dB SL. No significant difference was observed when 1000 Hz was compared with higher frequencies.

A rise in sensation level from 20 dB to 60 dB was associated with an increse of adaptation at all frequencies, but testing results for 250—1000 Hz at 40 dB SL were not statistically different from those measured at 60 dB SL. There was no further increase in adaptation from 60 dB to 80 dB SL in the frequency range 2000—6000 Hz adaptation seemed even to decrease

As a function of time, the adaptation tracing generally reached a maximum in all combinations of frequencies and sensation levels in 2½—3 min In certain cases (type II) however the increase at 60 and 80 dB SL continued for up to 3½ minutes when tested at 1000—6000 Hz.

The decline in loudness level was often accompanied with a change in the quality of the pure tone it acquired a resemblance to noise or was of lower pitch than the comparison tone The proportion of distorted stimuli increased with frequency so that at the four sensation levels it was less than 3 per cent for 250 Hz and 7 —84 per cent for 6000 Hz. Between 1000 Hz and 2000 Hz, the proportion of changed stimuli exceeded at all sensation levels, those that remained unchanged.

Prestimulatory balance amplitudes were regularly wider than the threshold amplitudes in all frequency SL combinations, excluding 250—1000 Hz. The excursion widths measured just before the end of the adaptation tracing were of the same order as the prestimulatory balance amplitudes — an indication of the fact, demonstrated by the aid of the comparison ear that the sensibility of the ear does not change in the adapted state The prestimulatory balance amplitudes showed a tendency to decline with increasing sensation level, but this was no longer detectable in the adapted ear

Poststimulatory mean threshold shift measured at the stimulus frequency with an interrupted test tone at the 60 sec. point varied from 2.6 to 4 6 dB The results were unaffected by the testing frequency and sensation levels, which is a sign of the physiological nature of stimulation in the normal hearing ear up to 80 dB SL.

In children as compared with adults, adaptation was of decisively different character In the group of 29 subjects aged 7 to 15 years (average 12.0 years) adaptation exceeding 10 dB (11—28 dB) could be demonstrated at 60 dB SL for 2000 or 4000 Hz in a total of 5 subjects, who were all among the oldest in their group (12—15 years) for 20 children tested the mean recorded at 3 min with a 2000 Hz stimulus was 1 0 dB S D 8 4 and for 23 children at 4000 Hz 0.3 dB S D 10 6 respectively At 2000 Hz the prestimulatory balance level was the same as in the normal group, at 4000 Hz somewhat higher Threshold amplitudes also were higher than in the normal group Thus they did not differ from prestimulatory threshold amplitudes, as in the main group In all other respects the results were consistent with the values found in the normal group

In a group of 10 subjects aged 56 to 70 years (average 64 0 years) adaptation at 60 dB SL from 250 to 2000 Hz was of equal amount as in the normal group At 3000 4000 and 6000 Hz, where the age linked threshold loss is more obvious, adaptation tended to decrease as the threshold loss increased, the

difference acquiring significance at 6000 Hz. At all frequencies the prestimulatory balance amplitudes, as well as the excursion widths of the adaptation tracings, exceeded the threshold amplitudes however the prestimulatory balance amplitudes did not differ from the excursion widths of the adaptation tracings, as they did not in the normal group. At each frequency tested the threshold amplitudes, prestimulatory balance amplitudes and the excursion widths of the adaptation tracings were all higher than in the normal group On the other hand, there were no differences in prestimulatory balance levels and poststimulatory threshold shift.

The testing procedure used is applicable to the study of all types of hearing loss, and its value in the differential diagnosis of perceptive hearing impairments is at present under investigation.

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From the Department of Otolaryngology
Massachusetts Eye and Ear Infirmary Boston

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A Clinical and Histopathological Survey

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CONTENTS

Paget's original observations are outlined and it is observed that osteitis deformans probably existed in ancient times.

The incidence of Paget's disease increases with age being one per cent in the fifth decade and eleven per cent in the ninth decade. The average age of the present group of 236 cases under review is 63 years. Osteitis deformans may be inherited as a sex linked recessive or as a simple Mendelian dominant gene. A family history is reported in 8 patients in the present study.

The paper reviews reports of deafness and vestibular disorders in this condition and the results of earlier tuning fork tests, audiological and radiological investigations in the present series is then presented. The incidence of tinnitus, vertigo and headache is outlined and complications are described, the most sinister being the development of osteogenic sarcoma.

Histopathological features of the disease process and its presentation in the temporal bone are described and the findings in temporal bones removed from patients suffering from osteitis deformans are then reported. The place of medical and surgical treatment is finally presented.

INTRODUCTION

"My head is twice as big as yours,
They therefore need must fit
William Cowper (1731–1800)

Paget's disease of bone was described in the latter part of the 19th century although it probably existed in ancient times. A skull from the Gallo-Roman era (Astre 1957) a parietal bone taken from an Egyptian tomb (Hutchinson, 1889) a femur from the neolithic period (Pales, 1929) several paleopathological specimens of American Indians (Denninger 1933 and Fischer 1935) were all thought to show features of Paget's Disease In 1885 Butlin examined specimens in the South Kensington Natural History Museum and found the skull of a Neanderthal man which had Pagetic changes.

Czerny in 1873 named the disease osteitis deformans, but it was Sir James Paget of St Bartholomew's Hospital, London, who correlated and described the clinical and pathological features In his first report in 1877 Paget analysed the case history of a man he had been following for 20 years (Fig 1) and reported four other cases of the condition He pointed out the distinction between this disease and the several forms of hyperostosis, osteoperiostitis and other diseases among which it had been confused Paget considered the disease to be an inflammation of bone and said, "I would suggest that for brief reference and for the present, it may be called after its more striking character Osteitis deformans" He then thought that a better name would be given when more was known of the condition Paget had seen an additional eighteen cases in 1889 of "a rare form of chronic inflammation of bones accompanied by change of shape, size and direction of the diseased bones" Although two other cases may have been presented earlier (Rullier 1812, and Wrany 1867) it was undoubtedly Paget who provided the most exact clinical picture Since his description no-one has suggested a better name for this condition than that of 'Paget's disease" for there is no conclusive evidence that the basic pathology is inflammation, nor is the disease in the majority of cases deforming. Thus the term "osteitis deformans" is perhaps rather less desirable than Paget's disease" Although there are many reports alleging the occurrence of this condition in animals (Hutchinson, 1889 Orr 1937 and White, 1922) the accounts given do not seem convincing and Paget's disease appears to be restricted to humans.

In an attempt to review some of the general features and more especially the effect of the disease on the temporal bone the records of 230 patients

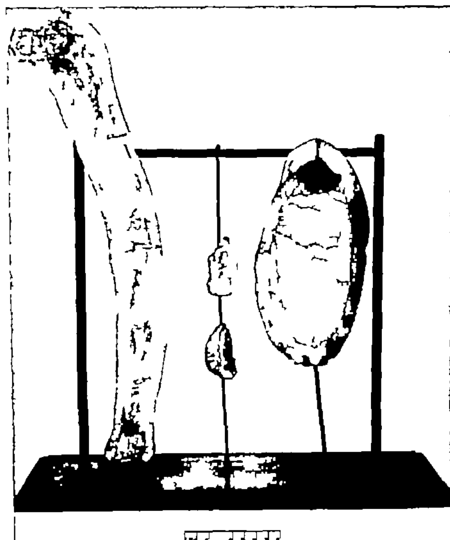


Fig. 1 The rigl l case Part f the ert x f the skull, pat ll nd tibia ffected by osteiti d formans. Thi specimen was presented t St. Barthol mew' Hospit l by Sir J mes P get d the phot graph is reproduced by ki d permission f the Curator f the M seum.

with Paget s disease who were attending or had attended the Massachusetts General Hospital or Massachusetts Eye and Ear Infirmary were examined More recently a further six patients were evaluated at St. Bartholomew's Hospital Twenty-eight of these patients had a full clinical examination carried out in 1965–6 It is the object of this report to briefly review the general features and published data and then to emphasize radiological audiometric and histo-pathological findings.

INCIDENCE AND AETIOLOGY

The real incidence of the disease was not fully realised until Schmorl s classical work in Dresden, on the anatomical pathology of the disease, was reported in 1930 This author found 138 cases of Paget s disease among 4614 autopsies of patients older than forty years, an incidence of three per cent A similar figure was obtained by Collins (1958) in a more recent survey in Yorkshire The incidence increases with age, and is still only one per cent in the fifth decade Hereafter it rises steadily reaching five to eleven per cent in the ninth decade In the patients reviewed in this report the youngest patient was thirty six years of age and the oldest was eighty nine The average age of the group was sixty-three years In rare cases, Paget s disease may start in young individuals and Ford (1960) quotes the case of Paget s disease beginning in a child of one year of age Males are thought to be affected more frequently than females (Porretta *et al* 1957 Pygott 1957) in the ratio of 4 3 although a few series have reported an equal sex influence (Gutman & Kasabach 1936 and Kay *et al* 1934) In the present review of 236 cases, 130 patients were male and 106 were female. Of these, there were two Negro males and three Negro females.

Since the description of the malady little progress has been made in the search for an aetiological factor Plausible and bizarre theories have been advanced since Paget described the disease as a chronic inflammatory process, although most of these now have only an historical interest Causative factors were thought to be infection syphilis or neurotrophic conditions. Endocrine glands malfunction have been blamed and it was suggested that an increase in growth hormone secretion might influence the local "enzyme mechanisms of calcification" (Rosenkrantz *et al.*, 1952) but immunological analyses for growth hormone carried out by de Deux chaines & Krane (1964) were found to be within the normal range.

Many of these theories were based on inadequate data and have fallen in the path of modern micro-analytical and histopathological advances. No None has stood the test of time and not one offers the explanation for the basic process, i e the anarchic destruction of bone without respect for the existing architecture accompanied by an extraordinary increase in all the elements of the local vasculature forming the periostial plexus (de Deux chaines & Krane, 1964)

The hypertrophy and hyperplasia of the vessels, and particularly of the arterioles, is such that one gets the impression that Pagetic bone is built at a different scale than normal bone (Rutishauser *et al* 1954) Whether this vascular hypertrophy is secondary to bone disease or whether it represents a primary cause of the disease is still in dispute Certainly the increase in vasculature is not secondary to increased bone metabolism since the oxygen saturation of the venous blood is high (Lequime & Denolin

FAMILIAL PAGET'S DISEASE OF BONE

5 Mrs AT aged 69 Bowing of legs noticed at age 57 now has advanced Paget's Disease, but is still ambulant. Serum alkaline phosphatase 140 K A

6 Mrs RT aged 67. In chronic sick hospital for over three years suffering from extensive advanced Paget's Disease.

Fig 2.

1955) The carotid flow in two patients with Paget's disease of the skull in this series being presented was measured with an electro-magnetic flow meter (Ojemann & Jain, 1963) It was found that the mean flow in the common carotid artery was increased to more than twice the average normal value Several authors have defended the hypothesis that Paget's disease is a primary vascular disease particularly Cone (1922) Brailsford (1953) and Moore (1951) The latter investigator is of the opinion that osteitis deformans is caused by sporadic failure of the autonomic nervous system which controls the blood vessels. Nevertheless, this hypothesis, as Moore himself admits, does not explain the absence of comparable changes in the adjacent soft tissues.

In 1955 Albright and Henneman produced evidence to indicate that large doses of A.C.T.H and cortisone damped down the disease process As Henneman *et al* (1963) later produced analogous changes with aspirin these authors speculated that osteitis deformans was of an inflammatory nature and perhaps an auto-immune disease of bone

The role of heredity has been stressed by Koller (1946) Ashley Montagu (1949) Galbraith (19[illegible]4) and McKusick (1960) Cases have been reported in identical twins (Aschner *et al* 1952 Dickson *et al.*, 1945 Koller 1946 Martin 19[illegible] and the disease has been found in four generations in one

instance by Dickson *et al* (1945) Figure 2 summarises the history of a family who were attending St. Bartholomew's Hospital Another English family in which six cases of Paget's disease have occurred in three generations has recently been reported by Jones and Reed (1967) Otosclerosis also has been reported in identical twins by Albrecht (1922) Shambaugh (1935) and Fowler (1947) Shambaugh's 1950 study of two thousand fenestrated patients yielded a positive family history in fifty four per cent. The mode of inheritance of otosclerosis cannot be established with certainty but the results of genetic analysis accord best with the hypothesis of a monohybrid autosomal dominant inheritance with a penetration of the pathological gene of between twenty five and forty per cent (Ruedi, 1963) While some wonder whether or not hereditary plays a significant role in Paget's disease (Gutman & Kasabach, 1936) others cite evidence that the disease is inherited as a sex linked recessive (Ashley Montagu 1949) or more probably as a simple autosomal Mendelian dominant gene (McKusick 1966) In the 236 cases reviewed, a family history of Paget's disease was discovered in eight patients Three of these patients had Paget's disease of the skull and an associated conductive deafness. Goodhill (1961) recorded the successful mobilisation of a stapes apparently partially fixed by Pagetic bone in a patient with a family history of osteitis deformans.

When a study is undertaken to determine the role of hereditary in a disease such as Paget's several difficulties are encountered By the time the disease appears, late in life parents are likely to have died and the siblings may be widely scattered geographically and the children will probably not yet be old enough to have developed the disease. In the second place Paget's disease is often subtle in its manifestations. A very high proportion of the affected persons are asymptomatic and the changes in bone are discovered only incidentally upon radiological examination made for unrelated purposes or possibly in a search for an explanation of an other wise obscure elevation of serum alkaline phosphatase

The occurrence of angioid streaks (two patients in this study) in a certain number of patients with Paget's disease, an anomaly in common with pseudoxanthoma elasticum as well as the occasional association of Paget's disease with pseudoxanthoma elasticum (Larmande, 1957 Moretti *et al.*, 1962 Shaffer *et al.*, 1957 Woodcock, 1952) suggested to McKusick (1966) the possibility that osteitis deformans may be a more generalised disorder of connective tissue

CLINICAL FEATURES

It is not within the scope of this paper to cover every aspect of Paget's disease and its complications but to outline and stress some of the features that may be of special interest to otolaryngologists. Although excellent

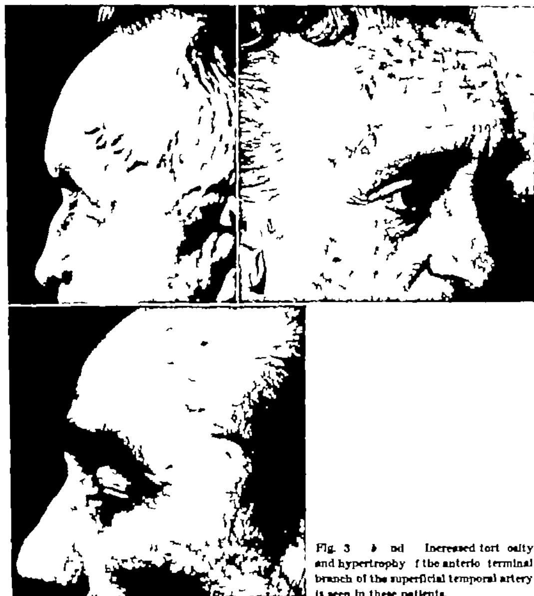


Fig. 3 *b* nd Increased tort osity and hypertrophy f the anterio terminal branch of the superficial temporal artery is seen in these patients.

reviews on large series of cases have outlined the general symptomotology (Dickson *et al* 194 Gutman & Parsons, 1936 Kay 1929 Newman 1946 Lackard *et al.*, 1901 Roberts & Cohen 1926 Rosenkrantz *et al.*, 1952 Sugarbaker 1940) the fully fledged clinical picture of a patient with an enlarged skull progressive kyphosis with shortening of stature is rarely seen However the subclinical nature of the disease as mentioned above i a relatively common form of bone disease The bones most frequently in volved are those subjected to the greatest stress. However Sugarbaker stated in 1940 that the skull, spine and pelvis, femur and fibula are the bones most frequently involved in the order named

Of the 236 p t nts that are being considered in this review 165 had skull inv l m t J get in his first report (18 7) stated that his original

4 / / ,



Fig 4 *T p l ft* P tient (B. D) i her twenties. *Bott m l ft* Same patient when 53 years f age *Right* Same patient when 73 years f g

patient "began to be somewhat deaf" and in his second paper in 1882 case number six was said to have impairment of hearing Although Leri in 1913 drew particular attention to the narrowing of all basal foramina, including the foramen magnum, in advanced Paget s disease of the skull the interest of otologists in the deafness of this condition was first aroused when Otto Mayer (1913) pointed out the similarities between the histological picture of Paget s disease and otosclerosis. By 1917 he was able to report a study of nine cases of the disease in which there was a hearing impairment and also reviewed two temporal bones with this condition

Nager in 1919 presented some evidence to explain the nerve impairment on the basis of involvement of branches of the auditory nerve by the forma tion of new bone Jenkins in 1923 examined nine patients with Paget s disease of the skull and found that the patients "were invariably deaf" In a study of four patients with Paget s disease Wyllie (1923) found that two pat ents had impaired hearing and noted that the hearing deficit was of the middle ear type Goldstein in 1926 reported the results of an extensive reveiw of some four hundred cases which had appeared in the literature He noted a history of impaired hearing in only twenty cases, an incidence of five per cent

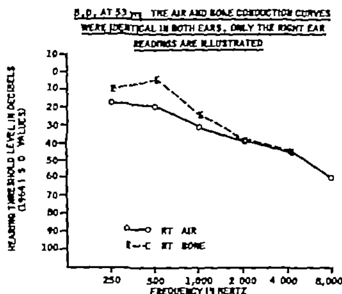


Fig. 5. Audiograph of the patient (B. D) seen in Fig. 4 at 53 years.

Further studies were later reported by Weber (1930) Brunner (1931) and Nager & Meyer (1932)

In 1934 Barth discussed four patients and reviewed twenty four cases from the literature including cases of both Paget s and von Recklinghausen s disease of bone In six patients there was inner ear deafness and in eight a combined middle ear and inner ear hearing deficit was noted. Barth found that the vestibular symptoms played an even greater role than the disturbance of hearing in these patients. As the individual cases were more critically examined from an otological viewpoint, the alteration in auditory function became more apparent In 1936 Lindsay & Perlman reviewed

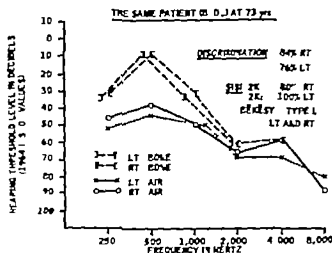


Fig 6 Audiograph of the patient (B. D.) seen in Fig 4 at 73 years.

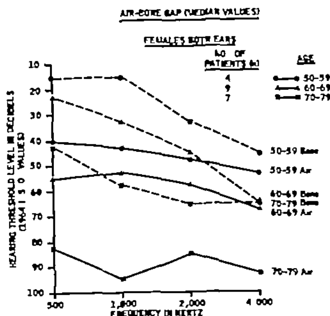


Fig. 7

twenty five cases of Paget s disease but only seven patients had skull in volvement In this latter group, three patients had tinnitus but there was no reported vertigo The most striking feature in four patients was a high tone bone conduction loss but in two of the patients a pinkish hue was noted on the promontory through intact tympanic membranes. Lindsay and Perlman postulated that these changes could have involved the annular

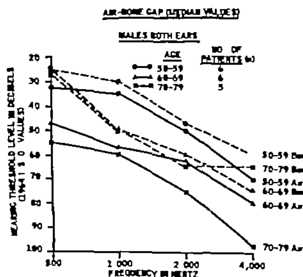


Fig. 8

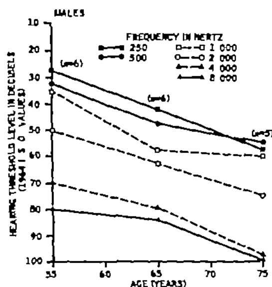


Fig 9

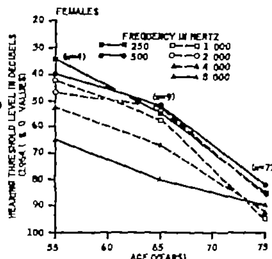
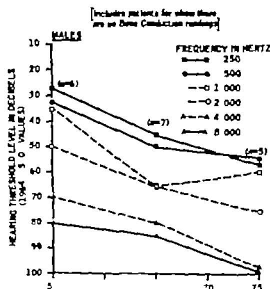


Fig 10

ligament or footplate of the stapes and might have accounted for the marked conductive loss in two patients. In ninety nine cases of Paget s disease Fowler (1937) reported deafness as the initial complaint in only three cases but in forty-one patients it was later the major symptom Tinnitus



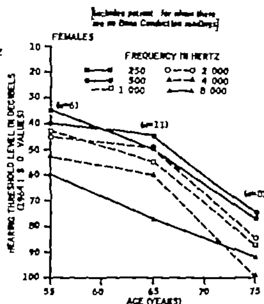


Fig 12

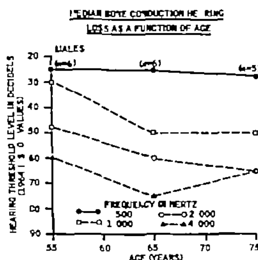


Fig 13

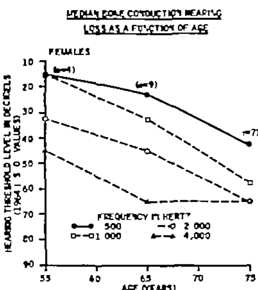


Fig 14

was present in ten instances and rotatory vertigo in twenty three Fowler concluded that the deafness usually occurring with Paget's disease is a "nerve deafness and affects the whole tonal scale or only the high tones"

In a review of 111 patients, Rosenkrantz *et al* (1952) found that bone pain was present in fifty five patients with Paget's disease defective hearing in fourteen, headaches in fourteen, vertigo in eight and gait disturbance in eight patients. Recently Clemis *et al* (1967) examined seventeen patients with proven Paget's disease and hearing loss. The hearing deficit was found most frequently to be of the mixed type The audiometric tests used did not suggest any evidence of a retrocochlear focus of the disease to account for the sensori-neural hearing loss.

Although small plaques of "calcification" in the auricle have been reported in Paget's disease, no similar pathology was noted in the group under review There is an occasional association of osteitis deformans with gout and three patients in this study were diagnosed as having this latter condition It seems likely that the earlier observations probably described tophi which occur in gout Kluyskens & Geldof (1965) have stated that abnormal curvature of the external auditory meatus and exostoses are frequently found in patients with osteitis deformans of the skull. Goodhill (1960) reported a patient with marked curvature of the external auditory meatus and F wl r (1937) noted bony overgrowth causing such marked obstruction th t n stoplasty was necessary A similar observation was menti ned b Sp w & Duval in 1967 and in the present study en patient w t l t have narrowing and mild tortuosity of the bony external audit ry m tu a a result of Pagetic changes. One patient had prominent bony ex st ses, but had been a very active swimmer for most of his life

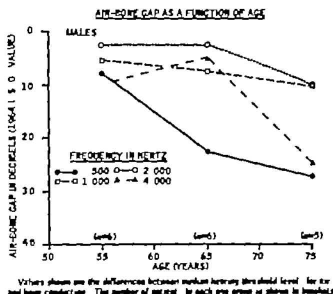


Fig 15

In many patients with Paget s disease of the skull prominence and tortuosity of the terminal branches of the superficial temporal artery was observed Figure 3 illustrates three patients with hypertrophy of the anterior terminal branch of the superficial temporal artery and although this condition is not pathognomonic of Paget s disease its occurrence associated with deafness should make the clinician aware of the possibility of osteitis deformans. Of the 236 patients studied that is, those with skull involvement and/or long bone involvement it was found that deafness was present in ninety seven patients. Of these fourteen complained of deafness but had no evidence of the disease on plain skull X rays.

On reviewing the earlier records of patients with Paget s disease it was obvious that little attention had been paid to the objective testing of viiith nerve function by the general physicians that had examined these patients. Nevertheless, tuning fork tests alone had been carried out in certain cases and in these patients with skull involvement it was found that a negative Rinne (512) was observed in eleven patients, positive Rinne in three and equal in one case

Nine patients had in the past air conduction audiograms carried out when they had attended the outpatients departments complaining of hearing loss. Full pure tone air and bone audiograms were obtained in forty patients, twenty-eight of these were examined in 1965 and 1966 and the readings from the remainder were obtained from the patients records and adjusted t 1964 I S O values.

One pat ent wh ha l been followed up for twenty years, had remarkable changes in h r f l ppe rance with the passage of time and this is illustrated Fig t I logram at the age of fifty-three (Fig 5) and her present he l l years later is shown in Fig 6

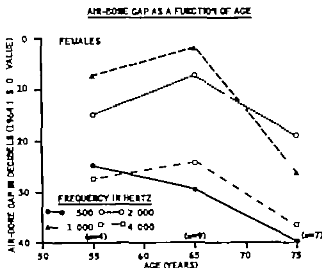


Fig 16 V l es hown are the differences between medi n he ring thresh ld lev l f air nd bone conducti n. The n mber of patient i each g group is hown in brackets.

The majority of patients examined were found to have a definite con ductive deafness in the low frequencies the air/bone gap being greatest at the 500 frequency The air/bone gap in the age group (*a*) 50–59 years, (*b*) 60–69 years, (*c*) 70–79 years for both male and female are seen in Figs 7 and 8 Bone conduction is seen to be better in the female samples and the air/bone gap is greater in this group Air conduction, bone conduc tion and the air/bone gap as a function of age is plotted in Figs 9 10 11 12, 13 14 15 and 16

SPEECH DISCRIMINATION SCORES

Speech discrimination scores were carried out in twenty four patients. Scores range from six per cent to one hundred per cent, the median values were

Age group	Number of patients	Median discrimination score
50–59	7	92 %
60–69	9	84 %
70–79	8	78 %

In all but ne pa ient similar scores were obtained in both ears but female patients usual y had a ten per cent better discrimination score than male patients in the same age group

Short Increment Sensitivity Index (SISI)

Twenty patients had this test carried out and in general the results agreed with Clemis *et al* (1967) in that at low frequencies the SISI scores were low but were high above 1000 Hz. At 4000 Hz all but one of the patients had SISI scores above eighty per cent

Békésy Audiometry

Eight patients had Békésy audiograms carried out and six tracings (twelve ears) were found to be of the Jerger Type I variety One patient had a Type IV pattern and one patient could not be fitted into the Jerger classification

TINNITUS

Thirty-one of the 105 patients with skull involvement experienced tinnitus In the majority (twenty) this was of a pulsatile nature and four patients noted that the frequency of the tinnitus was synchronous with their pulse Six patients had bilateral ligation of their external carotid arteries carried out in the past in an attempt to slow the progress of the disease and to aid the vascular headaches and four of these noted temporary abatement of the tinnitus It should be mentioned that a large number of patients with Paget s disease take aspirin as this relieves pain and like cortisone appears to induce a transient phase during which resorption is more decreased than bone formation One patient experienced tinnitus on high aspirin dosage which disappeared soon after the removal of the drug

HEADACHES

In advanced Paget s disease headaches were found to be a prominent feature and a constant finding in every patient with more than minimal basilar impression The most common seat of the discomfort was the occipital region but the temporal and frontal region was also a common localisation When the Paget s disease was mild, the headaches were not pronounced and often well localised In the earlier stages the headaches were intermittent but as Pagetic changes became more severe the headaches were constant

VERTIGO

Thirty five patients of the 105 cases with skull involvement experienced vertigo From the earlier records it was noted that one caloric test had

been carried out and this was normal but the diagnosis of Menière's disease had been given to five other patients although no caloric or other vestibular function tests were recorded. In the sub-group of twenty-eight patients seven patients complained of vertigo. Using the minimal caloric test of Kobrak it was found that two patients had bilaterally equal diminished caloric reaction and one patient had evidence of right canal paresis. The remaining caloric tests were normal. Patients with advanced osteitis deformans of the skull frequently have marked Pagetic changes in the cervical spine and this together with occipital condoyle invagination will produce symptoms of vertebrobasilar ischaemia.

COMPLICATIONS

The most sinister complication of Paget's disease is undoubtedly the development of osteogenic sarcoma. Eight of the patients under review developed sarcomatous changes. Coley & Sharp (1931) on reviewing seventy one cases of osteogenic sarcoma reported that twenty-eight per cent were associated with Pagets disease and in all their cases of osteogenic sarcoma of the skull there was existing osteitis deformans. Porretta *et al* (1957) found that of the one hundred and twenty-eight cases of skeletal sarcoma with coexistant Paget's disease reported in the literature twenty per cent were in the skull. There was only one five year survival in this series. It is thought that the risk of osteogenic sarcoma in Pagetic individuals over forty is thirty times greater than in normal patients (Price 1962) and in twenty per cent of the cases the osteogenic sarcoma is multicentric. Two patients in this present review had skull lesions resected and were reported earlier by Ojemann & Jain (1963). The first patient had a mass in the right fronto-temporal region and complained of diplopia and diminished hearing. A diagnosis of Paget's disease of the right femur had been made several years earlier. The second patient had complained of a swelling behind the right ear of six months duration and X ray examination revealed marked involvement of the skull by Paget's disease with bony overgrowth in the soft tissue in the right occipital region suggesting sarcomatous degeneration in this area (Figs. 17 and 18). It has been pointed out that branches of the superficial temporal artery can be hypertrophied and dilated in Paget's disease and when an angiogram is carried out hypertrophy of all the external carotid branches, particularly the meningeal superficial temporal and occipital arteries, and occasionally the ophthalmic artery are discernible.

A fifty five-year-old Negro woman entered the Ear Nose and Throat Department complaining of a swelling which had been increasing in size and was situated just in front of the right ear. On examination a pulsatile mass was seen [illegible] the ear and the superficial temporal artery ap-



Fig 17 Thi pati t had complained f sw lling behind th right ea Bony over growth (S) in th soft tissu i the occipital regi n, suggestive f sarcomatou dege era ti wa seen.

peared to enter the confines of the swelling Plain X rays of the skull and a right carotid angiogram was carried out and these indicated that the ex ternal carotid artery was hypertrophied The middle meningeal artery was found to fill an aneurysmal sac which originated in the anterior branch of the middle meningeal artery and this mass had eroded through both tables of the skull The superficial temporal branch of the external carotid artery passed over the surface of the swelling and was stretched by it. Following ligation of the external carotid artery the mass became firm and was obviously filled with blood clot and slowly reduced in size

Reports have been published describing Paget s disease in association with giant cell tumour and with lymphatic leukaemia. It seems that giant cell tumours co-existing with Paget s disease have a predilection for the bones of the face and skull These sites are strikingly different from those usually involved with true giant cell tumours unassociated with Paget s disea e N giant cell tumours of the skull or temporal bone were noted in this present er



Fig 18. Grossly dilated occipital artery (O) pplying the osteoge ic sarcoma.

X RAY APPEARANCE

In the present series, examination of X-rays showed that the initial process was predominantly osteolytic followed, often several years later by osteoblastic changes. In the skull areas of normal bone bone destruction, and bone repair were frequently noted in three adjacent areas Osteoporosis circumscripta was noted in the skulls of five patients. These skulls showed large sharply circumscribed areas of demineralisation and appeared con fined to the vault. Du Boulay (1965) has, however stated that the areas of translucency appear to begin low down in the vault, near the skull base and from there extend upwards He stated that commonly the first parts of the vault to be affected are the squamous occipital and the area around the pterylon.

The osteolytic phase eventually progresses into a more frequently observed type known as the biphasic or combined phase of osteitis deformans in which bone destruction and production occur simultaneously X rays of the skull in this phase showed demineralisation and ill-defined bony struc ture so that the tables, vessel markings and suture lines become less distinct There is a general thickening of the skull which loses its differentiation in the normal three tables, the inner table being less severely affected than the others. Small circular areas of increased bone density may be scattered throughout the cal aria giving the so-called cotton wool appearance in the



Fig. 21 Left tempor l ... P) can be een a tero-medi l t the cochlea

In more inferi r ... nt of the Pagetic involvement was seen to be in bone urr unding th c r tid canal The affected bone contained few steoclast

Two t mporal b nes fr m the sec nd patient (A K) a seventy five-year old male showed a m re advanced form of osteitis deformans. This patient was kn wn t have had Paget s disease for twenty years and five months before his death due t heart failure a suboccipital decompression of the spinal c rd and laminectomy f the first cervical vertebrae was performed for pres ure symp t ms due to bony overgrowth It was seen from his record that thi patient had bilateral progressive hearing loss for "many years and w re a hearing aid in the right ear Clinically this man s head was enlarged and X ray examinati n revealed extensive Paget s disease of the kull A general l w p wer view f the right temporal bone is illustrated in Fig 22 an l it is seen that the bony changes were most marked in areas best supplied with marrow tissue In serial sections the Eustachian tube reg n th petr us apex and mastoid regions were involved to the greatest extent The p sterl r margin of the left internal auditory meatus was enlarged t form a r unded bulge The labyrinthine capsules were due to thei ... marr wless structure least sensitive to attack The periostial lay being le t vascularised and provided with small marrow spaces showed th ... t c mplete rec nstructi n The middle enchondral layer possessing

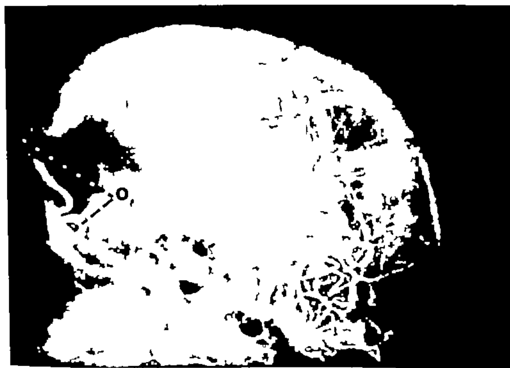


Fig 18 Grossly dilated occipital artery (O) supplying the osteogenic sarcoma.

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beginning and with time these areas increase coalesce and finally produce the classic mottled appearance of the "Tam O Shanter" skull Guillain & Aubrey in 1936 studied X rays of the temporal bone in Paget s disease and noted increased visibility of the semi-circular canals and labyrinth in the temporal bone in patients with this condition Lievre & Fishgold (1959) in their monograph on Paget s disease of the face and skull stated that whenever this condition affected the skull it nearly always affected the temporal bone In a study of twenty four patients with Paget s disease of the skull these authors found that sixteen patients had "subjectively some degree of deafness" However full audiological assessment was not carried out. In 1955 Vetrano suggested that there were four stages in the X ray and audiological picture and thought that a relationship existed between the auditory functional deficit and X ray signs However only one patient was studied Clemis *et al* (1967) has recently observed that of twenty-eight ears examined by tomography twelve revealed footplate thickening Two of these had no conductive loss, and this was thought to indicate a poor radiological-audiological correlation

In an attempt to study the X ray changes in the temporal bone in this condition ten patients with advanced Paget s disease and five patients with early signs of Paget s disease had tomograms taken of this area In the latter group it was seen that the initial osteolytic or resorptive phase was most pronounced in the petrous apex and in this stage the semi-circular canals and cochlea could be unusually well visualised Periostial bone was first attacked and later the denser enchondral bone of the otic capsule itself was involved The next stage of involvement appeared to be decreased visibility of the cochlea and lateral and superior semi-circular canals. The os icles in this stage of the disease appeared normal and in the majority of cases of patients with moderate Pagetic involvement the margins of the oval window niche were discernible More advanced cases of osteitis deformans had a complete blotting out of the otic capsule by the bony changes. Even though the labyrinth and cochlea could not be recognised in these patients on tomography they still had serviceable though poor hearing and there was no vestibular disorder In advanced Paget s disease it was im possible to assess the size of the internal auditory meatuses If stenosis existed it was probably caused by uncalcified new bone which did not cast a radiological shadow Six of the thirty temporal bones examined by laminography revealed an upward tilting of the petrous apices. In three of these the internal auditory meatuses were seen to point upwards, due to excessive softening and twisting of the base of the skull Examination of the right ear of one patient revealed that the handle of the malleus cast a very poor had w and seemed more translucent than the handle of the malleus on the other side (Fig 19) It was als found in this temporal bone that the val w ndow could not be readily identified and there seemed to be a fairly thick laver f bone around the lateral wall of the vestibule Audiological a se sment revealed a bilateral conductive deafness, the hearing loss in the



Fig 19 Ha dl f malleus (M) is more translucent in the right ea

right side being forty db greater than that of the left In another patient with advanced Paget s disease the mallei and incudes appeared to be greatly enlarged and were more massive on the right side (Fig 20) No marginal thickening of the footplate of the stapes was observed In general it was found that osteolytic changes could well be present in one temporal bone and the blastic phase predominant in the other temporal bone No unilateral involvement of the temporal bone was seen

The complication of occipital condoyle invagination or basilar impression was frequently seen in patients with moderate or advanced Paget s disease of the skull The angle between the basi sphenoid and the basilar portion of the occipital bone, normally 110–140 is increased in these patients. The foramen magnum is deformed the axis is occipitalised and the medulla is unusually low so that it and the upper end of the cervical cord can be compressed by the odontoid process In the total number of patients with skull involvement, varying degrees of basilar impression was noted in thirty five patients.

A patient may well have Paget s disease of the skull without any evidence of X-ray involvement. Normally 30–50 per cent of bone calcium in a local area must be altered before radiological changes are evident However if

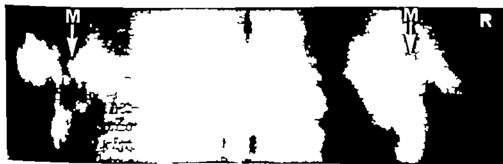


Fig 20 H d f the mallen (M) is more massive I the right ear

Strontium-85 scintiscans are employed bone disease in the skull can be detected early because of the demonstration of an accelerated mineral accretion rate which would not be evident initially by X-ray examination.

HISTOPATHOLOGY

In all vital bone changes there is classically an interplay of destruction and regenerative changes taking place and thus many features of similarity exist in the histological pattern of clinically different diseases, such as otosclerosis, Paget's disease and Recklinghausen's osteitis fibrosa.

When Mayer in 1917 gave the first account of the histological changes caused by Paget's disease in the temporal bone he described a case in which there was a bony bridge between the superior margin of the oval window and the footplate and noted that the newly formed red bone of Paget's disease was clearly separated by a cement line from the dark blue bone and cartilage of the footplate. Sections from three patients with Paget's disease were studied by Jenkins (1923) but the stapes were not found to be fixed. Nager & Meyer (1932) when studying a patient with this condition found calcification of the annular ligament that "almost fixed" the stapes and noted narrowing of the round window niche and some Pagetoid thickening on the promontory. In 1937 Anson & Wilson noted that in two patients with Paget's disease the newly formed bone had almost completely replaced the normal osseous architecture of the petrous bone but the stapedial footplate was found to be normal. Tamari (1942) on examining three patients with Paget's disease found that the newly formed bone was spongy in the petrous apex and in the mastoid process and more compact in the labyrinthine areas. The labyrinthine bone was profusely replaced by a compact, almost marrowless bone. Griffey (1960) in a report on the histopathological changes in the inner ear of an eighty-one year old patient noted that there was a mild bony protrusion in the medial aspect of the internal auditory meatus which might possibly have caused some slight pressure on the eighth nerve. "Involvement of the nerve to the macula" was stated to have occurred but the membranous labyrinth and cochlea were "free of any changes which can be attributed to Paget's disease". The ossicles in this patient were not examined.

Recently Wallner (1965) examined five temporal bones of patients suffering from Paget's disease of the skeleton and found that in three cases Paget's disease extensively involved the labyrinth. Every section (twenty-four) in the oval window area was stained and he reported in one temporal bone finding "newly formed partly lamellar partly younger bone piled up on top of the anterior portion of the footplate". It was thus apparent that the penetration of Paget's bone from the rim of the oval window into the anterior margin of the footplate was very small and localised but it

was not stated how many serial sections demonstrated this finding. Wallner also reported the histological examination of three footplates removed during stapedectomy of patients who had suffered from Paget's disease of the skull. Evidence of new bone formation was identified in two of the three footplates but definite histological Pagetic changes were only demonstrated in one of the footplate fragments.

In view of these interesting observations and the fact that only two patients out of the twenty cases previously reported indicated histological ankylosis of the stapes footplate by Paget's disease, a study of temporal bones taken from patients who had suffered from osteitis deformans was carried out. Eight temporal bones were examined in serial section and sections from three more patients were available for study. Of the eight serially sectioned temporal bones, no definite evidence of Paget's disease was found in three of these but in the remainder every consecutive section (twenty μ) was examined in the oval window area.

Different stages of osteitis deformans can appear in close proximity and the confusing variability of the diseased bone can usually be attributed to the combination of three labile factors:

1. The ratio of osteoclastic and osteoblastic activity which varies in any given area.
2. The speed of the regenerative and destructive process.
3. The frequency of local remissions and the ensuing resumption of active changes.

Local differences in the rates of osteoblastic and osteoclastic activities are responsible for the development of sclerotic and porotic areas, frequently observed in adjacent areas of the same bone. The mosaic pattern of Paget's disease may be attributed to two characteristics, the irregular and curved cement lines within the bony tissues and irregular notches and depressions on the surface of the spicules. The latter are caused by pronounced osteoclastic absorption and these structural features are caused by the simultaneous resorption of old and fully calcified bone and the deposition of new osteoid layers that calcify in a normal fashion. The resorptive process is carried to a certain point and then ceases. Later new bone is formed within this depression and a cement line remains to mark the point of application of new bone upon the old. At a later date, resorptive changes occur again so that the architecture of a given area is further disturbed. Increased vascularity and fibrosis of the intertrabecular spaces accompany this change in architecture.

The first temporal bone examined (MM) was that of a fifty nine year-old female who died of osteogenic sarcoma. There was no evidence of any otologic symptoms. Pagetic bone of the osteoblastic mature variety was found only anterior to the otic capsule (Fig. 21). The otic capsule and ossicles were normal as were the auditory and vestibular sense organs. The cochlear neuron population was also found to be within normal limits.



Fig. 21 Left temporal bone (M M) Pagetl bone (P) can be seen tero-medial t the cochlea

In more inferior sections the main extent of the Pagetic involvement was seen to be in bone surrounding the carotid canal The affected bone con tained few osteoclasts

Two t mporal bones from the second patient (A K) a seventy five-year old male showed a more advanced form of osteitis deformans. This patient was known to have had Paget s disease for twenty years and five months before his death due to heart failure a suboccipital decompression of the spinal cord and laminectomy of the first cervical vertebrae was performed for pressure symptoms due to bony overgrowth It was seen from his record that this patient had bilateral progressive hearing loss for "many years" and wore a hearing aid in the right ear Clinically this man s head was enlarged and X ray examination revealed extensive Paget s disease of the kull A general low power view of the right temporal bone is illustrated in Fig 22 and it is seen that the bony changes were most marked in areas best supplied with marrow tissue In serial sections, the Eustachian tube regi n the petrous apex and mast ld regions were involved to the greate t extent The posterior margin of the left internal auditory meatus was enlarged t f rm a r unded bulge The labyrinthine capsules were due to their alm t marr wless structure lea t sensitive t attack The periostial layer being l t vascularised and provided with small marrow spaces showed the m t c mpl te reco n truction The middle enchondral layer possessing



Fig 22. Right temporal bone (A h.) P tchy i vol ement by P get disease (*P*)

small and scanty vessels and cartilagenous enclosures resists for a time the rebuilding process. The changes of osteitis deformans were rather more active in the left temporal bone but very few osteoclastic cells were noted to be present. For the main part the Pagetic process was in the quiescent phase but areas of activity were noted especially around the Eustachian tube In no place was the endosteal bone breached. In both temporal bones serial sections through the oval window area did not show any bony fixa tion of the stapes and in the right temporal bone a thin strand of lamellar bone separated the posterior part of the annular ligament from the Pagetic process However the middle portion of this ligament was disorganised and replaced by a layer of loosely woven fibrous connective tissue similar in appearance to that filling the adjacent marrow cavity (Fig 23) Normally the shorter more resistant posterior ligament of the stapes allows this area to act as a fulcrum for a foot-pedal action when the stapes is rhythmi cally withdrawn and depressed into the oval window Greater excursions occur at the anterior end than at the posterior end, and the action is around the short axis of the foot-plate at low frequencies At high intensities the footplate no longer oscillates around the short axis but shifts to a rocking movement around the long axis. Involvement of the annular ligament by otosclerosis or by Paget s changes may manifest itself by a stiffness till on audiometric examination One very interesting feature noted in this patient s temporal bones was the presence of bilateral bony spurs from the antero-lateral aspect of the pitympanic walls to almost make contact with the

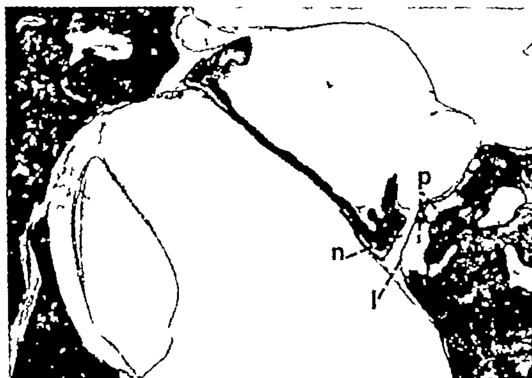


Fig. 23. Disorganisation of the posterior annular ligament by new fibrous tissue (N). A thin strand of lamellar bone (L) separates the Pagetic process (P) from the adjacent ligament.

heads of the mallei (Figs 24 and 25) These projections showed characteristic changes of osteitis deformans but no fixation of the mallei were noted Bony projections in this position are not uncommon and occasionally when well developed the malleus head is fixed by a rigid bar to the outer attic wall (Davies, 1968) In the past in Paget's disease the conduction deafness has been assumed to be due to interference with the movement of the stapes by the disease but many surgical reports have indicated that the foot plate area was found to be normal at exploratory tympanotomy Needless to say it is essential to palpate the handle of the malleus when a conduction hearing deficit is being surgically investigated

A moderate loss of ganglion cells in the basal turn of the cochlea was seen and the organ of Corti was atrophied in the extreme basal end of the cochlea but elsewhere appeared normal This mild degeneration of sensorineural elements in the basal turn could not be regarded as being a major contributory factor in the deafness experienced by this patient. There was moderate t severe atrophy of the stria vascularis in a large part of the middle and apical turns of both ears but the vestibular sense organs appeared normal bilaterally

The third set of temporal bones examined were removed from an eighty four year-old w man (A B) who died as a result of a fracture of the left



Fig. 24. Right temporal bone (A. K.). Paget projections (S) arising from the epitympanic wall and lying in close proximity to the head of the malleus (M malleus, I incus).

femur which was involved by Paget's disease. The patient had no complaints referable to the hearing or vestibular systems. The histopathological changes in the temporal bones were almost identical bilaterally. The entire bones, including the ossicles, were involved in Paget's disease. The otic capsules were irregularly eroded by a clearly defined layer of Pagetic bone (Figs. 26 and 27) and in the region of the superior canal in the right temporal bone the endosteum itself was involved and breached by fibrous connective tissue (Fig. 28). The greatest resistance to Paget's disease is normally shown by the endosteal layer, the modiolus and the macula cribrosa, for these parts are built by ossification of connective tissue and the resistance against replacement or reconstruction seems to a great extent to be dependent upon their vascular supply.

No anastomotic connections normally exist between the blood vessels of the bony otic capsule and the vascular system of the membranous otic labyrinth. Ruedi (1965) has stated that when a rapidly growing focus of otosclerosis has broken the capsular endosteum, such as in the region of the spiral ligament, new connections are formed between the otosclerotic vessels and the spiral veins of the membranous labyrinth as they pass through the spiral ligament, and venous blood from the otosclerotic mass then passes through the membranous labyrinth into the venous system of the inner ear. He observed that the stria vascularis reacts to this disturbance



Fig. 20 Right temporal bone (P K) A more active variety f P get dise ses I d m- on trated. The oral window area I clea but breaching (B) f th dosteum ca be seen in the basal turn f the cochlea.

of circulation with a swelling of the afferent and efferent capillary loops and epithelial hyperplasia in the region involved In this patient s left tem poral bone the endosteum was seen to be breeched in the apical turn of the cochlea but although this area of the bone contained a large number of vessels, no venous shunt was discernable and the stria vascularis ap peared normal The resorptive process in this temporal bone had tem porarily ceased and very few osteoclasts were seen In both bones a good population of hair cells in the cochleae were apparent and the spiral gang lion cells and vestibular ganglia appeared normal The serial sections of the foot plate region did not reveal any stapedial fixation although patchy Page-tic changes were noted in the malleus, incus and stapes suprastructure

A more active variety of Paget s disease of the temporal bone (P K.) is illustrated in Fig 20 A full series of sections was not available but in those examined the otic capsule was almost completely replaced by ab-normal bone extending down to the endosteum of the apical and middle turns and the internal auditory meatus appeared rather narrow The pres ence of a large number of multinucleated osteoclasts and osteoblasts and the presence f marked fibrotic changes in the marrow demonstrated the obvious tivity f the lesions Apart from mild post mortem changes the cochlea an l v tibul r labyrinths appeared unaffected. In the limited



Fig. 30 Right temporal bon (S.O.) Th endosteum h been breached area nd there is lmost complet replacement f the enchond l rge pneumatic cell which had extended from the post ro- petrou bone can be seen here t ext nd t the lev l of the i ternal

number of sections available from this patient no every was noted in the foot-plate area.

Temporal bone sections were a ailable for study with Paget s disease It was observed that there was of the otic capsule with osteitis deformans A large bone had been attacked by Paget s disease and in sev teum had been replaced However no invasion of labyrinthine spaces were noted, and the middle ear by the disease process A large tract of pneumatic superior region of the petrous bone was seen to level of the internal auditory meatus (Fig 30) autolysis was severe the organ of Corti was identified It was estimated that there was a loss of the spiral ganglion cells in the lower basal turn and and Scarpa s ganglion appeared normal

Three sections were available for study in an fifty four year old) and examination revealed replacement of temporal bone by Paget s disea breaching of the ndosteum layer and the



Fig 31 Right temporal bone (P K) widespread ost itis d f rm s. The endosteal l y is I tact nd the neural l m t re within normal limits. P getic changes rround the l window area but the footplat i free f the disease process.

meatus and sense organs appeared normal The spiral ganglion and Scarpa s ganglion also were within normal limits (Fig 31)

Allen & J hn (193) first discussed fissure fractures in I aget s disease They f und that patients with osteitis deformans were more prone to fractures of the femur when this bone was involved by the pathological process In n rmal temporal bones micro-fracture stress lines are fre-



Fig 32. Right temporal bon This bone w removed from patient with widespread ost itis deformans. N Pagetic ha ges can be seen i the icinity f the transverse fract re lin

frequently found in the otic capsule and are without significance (Mayer 1930) Fowler (1937) however stated that the bone growth of Paget s disease actually fractures the labyrinthine capsule so as to deform the cochlea and so affect the end-organ of hearing, a well as the nerve trunk. *A temporal bone from a patient who had died as an indirect result of a* fracture through Paget s disease of the femur was examined, and a fracture was also seen in the temporal bone (Fig 32) However it was evident that although no past history of cranial trauma could be obtained from the patient s relatives, this fracture line was old and no evidence of Pagetic changes were noted in the surrounding areas. The fracture had involved the inferior division of the vestibula nerve and there was associated degeneration of this nerve trunk.

Nager & Meyer (1931) stated that it was sometimes impossible to distinguish between osteitis fibrosa and osteitis deformans in the temporal bone and occasi nally today it is essential to examine other parts of the bony skeleton to ascertain the exact nature of the type of metabolic disorder present In Paget s disease there is normally a more complete obliteration of the original osseous trabeculae *than is the case in bone that* has been involved by secondary hyperparathyroidism Fig 33 is a lower power view of a patient with secondary hyperparathyroidi m and superficial similarities

for the treatment of Paget s disease of the temporal bone and otosclero is fraught with danger if patients are not carefully observed and critica assessed

SURGICAL TREATMENT

It was mentioned earlier that six patients had their external carot arteries ligated in an attempt to get rid of the vascular headaches and sl down the progress of the bone disease However only temporary subjecti relief was noted

Early attempts to mobilise the stapes were carried out with limit success (Gaillard *et al.*, 1959 Goodhill, 1961) Wallner (1963) report five patients with Paget s disease who underwent stapedectomy and stat that three of the five had a useful improvement for at least one year Ho ever the air bone gap was not closed in any of these patients In the prese series, stapedectomies were carried out in two patients with osteitis d formans. In the first patient the air bone gap was closed in frequenci below two thousand cycles per second but a gap of fifteen decibels existe in the high frequencies. The second patient had advanced Paget s disea of the skull and an extremely narrow and tortuous external meatus. exploratory tympanotomy the malleus handle was found to be stiff o palpation but mobilised easily when firmer pressure was applied I ressu on the footplate did not produce a round window reflex but there was r evidence of any bony fixation of margins of the footplate A total stapede tomy was carried out and histological examination of the stapes reveale the mosaic pattern of osteitis deformans to be present in the footplate an in the head of this bone (Fig 34)

Though there was subjective improvement a month after surgery audi metric assessment only indicated a gain of fifteen db and an air bone ga of fifteen db still persisted In retrospect it would have be n far wis to have exposed the epitympanum and removed the head of the malleus, a it now seems likely that epitympanic fixation has recurred

SUMMARY AND CONCLUSIONS

Any patient who complains of obscure pain in the head or exhibit an unexplained cranial nerve palsy should have a plain X ray examination the skull carried out to exclude osteitis deformans. Strontium 85 scintiscan can detect Paget s disease by the demonstration of accelerated mineral ex cretion rates before any evidence of X ray change is noted Normally thi to fifty per cent bone calcium in a local area mu t be affected before rad logical change are discernible

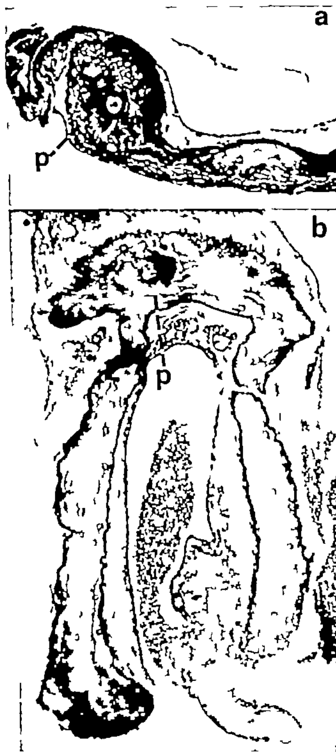


Fig. 34 Change f ost itl deform ns (p) i th footpl t ( ) and i the head f the st pes (b) re indicated.

The recognition of sarcomatous changes in Paget's disease of the skull is often difficult but a change in the nature of chronic bone pain is suggestive. The development of a swelling is a later but more obvious sign. Generous biopsy of any suspicious area is mandatory for an early diagnosis. Although osteogenic sarcoma is the commonest, fibrosarcoma, chondrosarcoma or giant cell sarcoma are occasionally discovered.

A prominent anterior terminal branch of the superficial temporal artery was frequently observed in patients with Paget's disease and was related to the increased vascularity in this condition. The existence of this enlarged artery is by no means pathognomonic of osteitis deformans but its presence together with a conductive deafness, should make the otologist aware of the possibility of Paget's disease as an aetiological factor.

Of the 236 patients in this study a family history of Paget's disease was noted in eight patients. Undoubtedly this figure would have been higher if all asymptomatic relatives had been subjected to a skeletal survey.

Deafness in early Paget's disease in the majority of patients was of the conductive type and the air bone gap was greatest at 512 Hz. As the disease progressed the air bone gap widened in the lower frequencies and a sensori neural hearing loss became more prominent in the higher frequencies. No evidence of a retro-cochlear lesion was demonstrated as a cause of the deafness. Caloric testing of labyrinthine function revealed only depressed function in a minority of the patients. Paget's disease can produce advanced changes in the cervical spine and this, together with occipital condoyle invagination, can result in vertebro-basilar ischaemia.

Tomographic examination was found to be an useful adjunct in assessing the size of the ossicles and the oval window area. One patient was noted to have a massive malleus head. However no definite correlation was found between the X-ray findings and the audiological picture. It was impossible in advanced osteitis deformans to accurately assess the size of the internal auditory meatuses for the presence of sclerosis as uncalcified bone casts a poor radiological shadow.

Eight temporal bones were examined in serial sections and sections from three more patients were available for study. Every consecutive section (20 μ) in the oval window area was carefully inspected. Pagetic changes were most marked in areas best supplied with marrow tissue. The Eustachian tube region, the petrous apex and mastoid areas were involved to the greatest extent. The labyrinthine capsules were, due to their almost marrowless structure, least sensitive to attack. The periosteal layer, being best vascularised and possessing rather small spaces, showed the most complete reconstruction. The middle enchondral layer having small and scanty vessels and cartilaginous enclosures showed more resistance to the rebuilding process. Endosteal bone was breeched in several bones but no constant pathological changes were observed in the stria vascularis and no venous shunts were discernible.

Bony fixation of the stapedial footplate was not noted but changes were

seen in the annular ligament in one temporal bone which were induced by adjacent Pagetic bone Changes of osteitis deformans were observed in many of the ossicles examined and in one patient bilateral Pagetic bony spurs were seen, in the attic region to be in close proximity to the heads of the mallei

Epitymanic fixation of the malleus and incus by the disease process could account for the persistent mild conductive hearing loss following an otherwise successful *stapedectomy* in these patients.

The methods of treatment used were discussed and the dangers of fluoride therapy were stressed

ZUSAMMENFASSUNG

Das Auftreten und die Aetiologie von Pagets Krankheit wird diskutiert. 236 P tienten mit Ost itis Deformans wurd n untersucht und wo möglich audiologische und radiologische Proben wurden gemacht.

Eine Luft/Knochen Kluft wurde baervlert in den untersten Frequenzen bei den meisten Patienten und dies war am klarsten bei 512Hz.

Als die Krankheit fortschritt die Luft Knochen Kluft wurde grösser in d n untersten Frequenzen und in den höheren Frequenzen ein bl ibend Gehörwahrnehmungsbestandteil wurde kon tatiert

Schwindeln war nicht eine orragende Eigensch ft aber wirbeloba llare Blutleere könnte verursacht werden durch Beteiligung der Halswirbelsäule wie auch durch Invagination der Hinterhauptprotuberanz. T m gramme des Schläfenknochen gaben unbefriedigende Resultate bei der Feststellung b Verengerung in den innern Gehörgängen stattfand weil verkalkte Knochen wertlose Röntgenschatten werfen. Abweichungen in der Ovalf nsterzone und in den Hämmern kamen öfters zu Licht im ersten Stadium der Krankh it

Histologische Untersuchungen von 11 Schläfenknochen produzierten gar k inen durchschlaggebenden Beweis der Steigbügelfixierung wegen Fortschrittes der Krankhe t epitympanische Auslauf jedoch, resultierend von der P gets Krank h it, *wurden bei einem Patient n in de Nahe d r Hammerknöpf gefunden.*

Die verschied nen Variation n d r Behandlung kamen unter Diskussion und die Gefahren der Fluorverbi dung Therapi wurde skizzi rt

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THE VASCULAR ANATOMY OF THE COCHLEA IN THE GUINEA PIG AND IN MAN

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CONTENTS

I. INTRODUCTION

Although the cochlea has been extensively investigated, there remains a lack of information about the vasculature. The survey literature provides only short and sometimes inadequate statements concerning the vascular anatomy. Furthermore, the information available in individual research works is incomplete and sometimes conflicting. This confusion arises from the use of different species of animals, different techniques for the demonstration of the vascular anatomy and different nomenclature. Photographs of variable quality and drawn figures which are in varying degrees schematic have increased the difficulty of arriving at a clear concept of the vasculature. Only little quantitative data are available on vascular dimensions, occurrence, distribution, and the general vascular arrangement.

The physiological mechanism responsible for the production of the labyrinthine fluids is still not settled. Although the capillary sections of the vascular bed are obviously involved in both production and absorption of the intralabyrinthine fluids, the detailed descriptions of all capillary networks necessary for the understanding of these problems are still incomplete.

The functional integrity of the cochlea obviously depends upon the maintenance of an adequate blood supply. Clinical experience indicates that perceptive deafness is often attributable to pathological conditions of the cochlear vessels. Of necessity, hypotheses attempting to account for pathological conditions of the circulation of the inner ear and their treatment have often been based upon insufficient knowledge of the vascular anatomy of normal physiological conditions, and of the pharmacological mechanism of the therapy used.

The aim of the present investigation was:

— to provide a critical survey of the literature concerning the vascular anatomy of the cochlea.

— to refine and simplify the method for the demonstration of cochlear vessels.

— to investigate the vascular anatomy of all areas of the cochlea in the guinea pig and man.

— to obtain quantitative data on vascular dimensions and relationships.

— to suggest how certain physiological processes and clinical disorders may be correlated with the vascular anatomy.

Comment

The advantage of direct *in vivo* observation of the vascular areas is that hemodynamic processes as well as the vascular anatomy may be observed and filmed under "normal" as well as induced pathological conditions. The limitations are that only small parts of the external wall in the apical turns of the cochlea can be observed after a very delicate preparation. The method is thus not appropriate for anatomical studies of all areas of the cochlea. Instead, this technique has generally been used in connection with vascular experiments. The use of serial sections gives access to all regions of the cochlea. However in addition to being time consuming and laborious, it provides only a relatively poor stereo conception. The advantages of abandoning this technique in favor of surface preparation methods have been emphasized by Engström et al (1962, 1966) Staining of the intravascular blood gives results similar to the injection of contrast medium, but requires the presence of blood corpuscles in all vessels, which is not always the case. Photos obtained by this technique clearly demonstrate the presence of partially uninjected vessels.

From previous results it appears that the injection of contrast medium is perhaps the most appropriate technique for studies of the vascular anatomy It can be carried out in cadavers or in living animals under general anesthesia. The method allows a good stereo conception and photographic registration of all parts of the cochlea. The high contrast obtained with injected vessels is probably superior to other techniques for visualization of the vascular anatomy

TABLE I

Previous investigations of the vascular anatomy of the cochlea in mammals

Guinea pig in vitro	Schwalbe, 1887; Nabeya, 1923 Agazzi, 1948; Smith, 1951; Wüstenfeld & Kühnert, 1964
Guinea pig in vivo	Seymour 1954; Weille et al., 1954; Weille, 1955; Perlman & Kimura, 1955 a, b; Naumann et al., 1958; Perlman et l., 1959 a, b; Nomura, 1961; Perlman & Kimura, 1962; Perlman et al., 1963; Bonaccorsi & Sambuco, 1964 Tamoo & Perlman, 1964 1965; Costa, 1968.
Rat	Asai, 1908 Nabeya, 1923
Dog	Eichler 1892; Asai, 1908 Nabeya, 1923
Cat	Nabeya, 1923; Smith, 1954
Man	Eichler 1892 Siebenmann, 1894; Nabeya, 1923; Agazzi, 1949 Scuderi & del Bo, 1952 Smith, 1954; Charachon, 1961 Levin, 1964
Other mammals	Ibsen — different mammal including man, 1881 Shambaugh — embryo pig, 1903; Nabeya — rabbit, monkey 1923; Agazzi — rabbit, 1949

B *Survey of the vascular anatomy*

The authors and publication dates of detailed investigations of the vascular anatomy of the cochlea in mammals are compiled in Table I Pioneering investigations were performed by Ibsen and Schwalbe. The work of Ibsen was carried out in 1841 but was not published until 40 years later after his death. At the end of the last century Eichler and Siebenmann performed the first investigations of the vascular anatomy of the contrast injected human cochlea and presented detailed drawings. Eichler further included a thorough survey of the previous literature on the subject. A subsequent comprehensive study by Nabeya (1923) of the vascular anatomy of different mammals is probably the most correct general survey available today However this work is particularly focused on the larger vessels and, in minor degree, on the capillary regions.

More recently Smith made a detailed analysis of the capillary regions of the cochlea in the guinea pig, the cat and man. Smith's papers provide the first thorough descriptions of the vascular arrangement in the external wall and the spiral lamina. Nabeya, Smith and Scuderi & del Bo published the first photomicrographs of these areas. Scuderi & del Bo's paper also contains a survey of the literature on the main vascular supply and the capillary regions. Their investigations are focused on the latter and on the vessels in the modiolus. The

Fig 1 Schematic view of the vascular system in the membranous labyrinth (from Nabeya, 1923 Original in color)

latest papers on the subject have in general confirmed the findings of previous authors without further contributions of importance (Charachon, Wüstenfeld & Kühnert, Levin)

The survey of the vascular anatomy below is a compilation of the often conflicting information given by the various authors with a selection of predominating viewpoints.

The basilar the anterior inferior cerebellar and the labyrinthine arteries

There is general agreement that the inner ear is supplied by only one main vessel the *labyrinthine* artery (also called the internal auditory artery) The origin of this vessel is the following vertebral — basilar — anterior inferior cerebellar — labyrinthine artery (Fig 1) In textbooks the distribution of these vessels is described as a symmetrical and fixed system. There are, however great variations in their size, length, course and ramification (Stopford, 1915 Konaschko 1927 Charachon, 1961) Variations have been shown in the origin of the labyrinthine artery which has led to confusion. Most commonly the labyrinthine artery is a ramification of the anterior inferior cerebellar artery which is one of the first large branches of the basilar artery (Watt & McKillop, 1935 Nabeya, 1923 Sunderland, 1945 Charachon, 1961 Levin, 1964) The branches supply a part of the pons, the labyrinth and parts of the cerebellum. However the origin of the labyrinthine artery can vary It can arise from the vertebral or basilar arteries, from proximal parts of the anterior inferior cerebellar artery or more distally in the inner acoustic meatus. When the labyrinthine artery arrives in the inner acoustic meatus it divides to form the *anterior vestibular artery* and the *common cochlear artery* The latter in turn soon divides into two terminal branches the *vestibulo-cochlear artery* and the *cochlear artery* (Fig 1) The various names used by different authors for these vessels are given in Table VIII page 124 No great differences seem to exist between the guinea pig and man concerning the distribution of these vessels.

Comment

There is reasonable agreement concerning the main vascular supply to the cochlea. The variations of the ramifications can be explained by differences between species and by individual variations within the same species. Unfortunately the nomenclature used and the descriptions of the vessels in the inner acoustic meatus vary considerably giving rise to difficulties in the interpretation of the subject.

The posterior vestibular artery in the guinea pig and the vestibulo-cochlear artery in man

These two names are used for the same vessel (Table VIII, page 124)

Guinea pig The *posterior vestibular artery* ramifies from the common cochlear artery at the cochlear base. It takes an oblique course along the central margin of

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Fig. 1. Schematic view of the vascular system in the membranous labyrinth (from Nabeya, 1923. Original in color.)

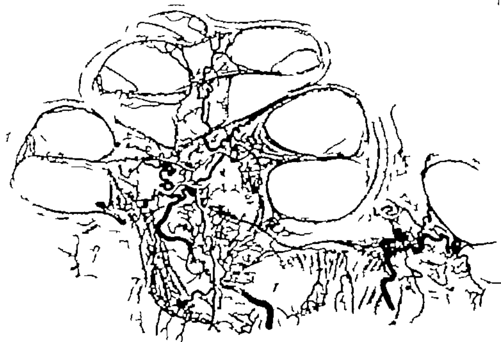


Fig 2. Drawing of radially-sectioned human cochlea showing the larger vessels in the modiolus and in the scalae Arteries appear darker than the veins (from Siebenmann, 1894 Original in color)

vestibulum and parts of the labyrinth, and the other supplying the rest of the cochlea. According to Siebenmann three branches from the artery take part in the formation of the tractus spiralis arteriosus which runs a serpentine course around the modiolus (Fig 4) Siebenmann considers the primary branches to form arcades. The description of the arterial system as presented by Siebenmann is not always clear Nabeya maintains that the trunk of the cochlear artery takes part in the formation of the tractus spiralis arteriosus and supplies the capillary regions of the cochlea, except the most basal parts. By the term tractus spiralis arteriosus Nabeya and Siebenmann mean that the main stem of the two arteries which supply the cochlea form a continuous route. Nabeya and Charachon could not always demonstrate the cochlear artery which was replaced in these cases by the cochlear branch of the vestibulo-cochlear artery

Comment

The cochlear artery is the most important in the cochlea. In man it is sometimes substituted for by the cochlear branch of the vestibulo-cochlear artery Opinions regarding its ramifications, the existence of "glomeruli" arcades and of the tractus spiralis arteriosus are varied. There is general agreement, however that the branches supply the capillary regions of the whole cochlea, except the most basal parts.

Capillary regions[1]

Modiolus

A compilation of published data indicates that there are three capillary regions in the modiolus of the guinea pig and man (Eichler Siebenmann, Nabeya, Levin) 1 in the acoustic nerve, 2. in the spiral ganglion, 3 in the bony modiolus wall (Fig. 2) The capillaries forming a dense network in these regions are branches of the cochlear artery or of its primary branches (Nabeya, Smith, Charachon) or from the tractus spiralis arteriosus (Siebenmann) Basally the capillaries derive from the vestibulo-cochlear artery in man and the posterior vestibular artery in the guinea pig

Apically the terminal branches of the cochlear artery end in an irregular capillary network connecting with the venous side. The capillaries in the modiolus are considered to drain to the spiral veins. The capillaries are endothelial walled tubes with occasional smooth muscle cells (Smith)

Comment

The capillary regions are situated in the nerve, the ganglion and the modiolus wall where they form irregular networks. These are supplied by the artery in the modiolus or its primary branches or the tractus spiralis arteriosus, and they drain to the spiral veins. There appear to be no great differences between the guinea pig and man in the distribution of these vessels.

Spiral lamina

Ibsen was the first to have a modern concept of the vasculature of the spiral lamina. He demonstrated that the vessels in this region form anastomosing loops and that small branches radiate over the whole spiral lamina to veins in the spiral ligament. Today's concept is that radiating arterioles penetrate the osseous spiral lamina at quite regular intervals (Fig 2, 3 4 5) They first run convoluted but straighten further peripherally The radiating arterioles derive from the tractus spiralis arteriosus (Siebenmann) or from the cochlear artery or its primary branches (Siebenmann, Nabeya, Smith) or from the vestibulo-cochlear artery basally The radiating arterioles gradually lose most of their muscle coating and are reduced to capillary size when they enter the spiral limbus (Smith)

The spiral vessels in the spiral lamina drew attention long ago and were the subject for much discussion in the latter half of the last century The number of spiral vessels demonstrated in this region has varied from none to four in the same and different animal species (the reader is referred to the discussions by Eichler Scuderi &. del Bo and Charachon) There has been general agreement that the spiral vessels and the vessels in the spiral limbus are terminal loops of capillaries running spirally for short distances. They are alternately supplied

[1] The vascular nomenclature is given on page 29



Fig 3 Drawing of transverse section of the spiral lamina from the basal turn of the guinea pig cochlea. I general the veins ppear straighter and darker in the figure (from Nabeya, 1923 Original in color). ACP = A. cochleae propria, VLP = radiating venules f the spiral lamina.



Fig 4 Drawing of transverse section of the spiral lamina from the basal part of the basal turn of the human cochlea. The veins are represented as being somewhat straighter and lighter than the arterioles (from Siebenmann, 1894 Original in color).

by arterioles and drained by venules (Fig 3 4) Viewed from above the capillary arcades formed by the spiral vessels are seen to be elongated in a spiral direction. At least in the basal turn this arrangement looks like a continuous vessel and has been considered to be so In the guinea pig there are two spiral vessels, one under the tunnel of Corti and one in the tympanic lip In addition, the capillaries in the spiral limbus form a spiral network (Nabeya, Smith) (Fig 3) In man the spiral vessel in the tympanic lip is less evident and is replaced by two zones of capillary networks in the tympanic lip and the spiral limbus (Nabeya, Smith, Scuderi & del Bo) (Fig 4) Apically the spiral vessels are much less continuous. The capillaries are endothelial walled tubes with occasional smooth muscle cells (Smith) and the vessel below the tunnel of Corti appears to contain fewer contractile elements than the other capillaries (Smith Spoendlin, 1957)

In general no vessels have been demonstrated in adult guinea pigs or man in the vestibular membrane, the tectorial membrane, or in the zona pectinata. Siebenmann, however demonstrated capillaries in the vestibular membrane in human embryos of the sixth fetal month. Siebenmann and Levin are the only ones claiming to have demonstrated vascular connections between the membranous cochlea and the surrounding bone.

Comment

The vascular system of the spiral lamina is arcadic, i.e. arcades connect centrifugally radiating arterial branches with centripetally radiating venous branches. The arcades form spiral vessels under the tunnel of Corti and in the tympanic lip or capillary zones in the tympanic lip and the spiral limbus. The exact localization of the spiral vessels has been much debated and there may be differences between the guinea pig and man. Otherwise the two species seem to be largely similar

External wall

In addition to the supplying radiating arterioles and the draining collecting venules, a compilation of modern authors recognizes four vascular areas in the external wall (Fig. 5 6 7)

Capillaries of the apical parts of the spiral ligament.

The stria vascularis of the scala media.

The vessel of the spiral prominence

Anastomoses in the deeper parts of the spiral ligament.

The *radiating arterioles* have been described as deriving from three sources a) the primary branches of the cochlear artery (Eichler — man, Nabeya — guinea pig and man) b) the tractus spiralis arteriosus or the arcades (Siebenmann — man) and c) the vestibulo-cochlear artery in the basal turn in man or the corresponding posterior vestibular artery in the guinea pig.

The radiating arterioles are given off at right angles and at regular intervals in the modiolus and form some anastomoses with each other centrally The

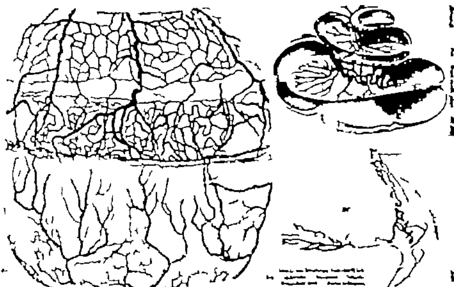


Fig. 5. Left: external wall of the human cochlea. Right, above: radial section of the human cochlea showing the course of the artery running around the modiolus. Right, below: radial section of the scala media in the dog (from Eichler 1892. Original in color).

arterioles follow a tortuous course through the bone but more peripherally they straighten. In general the arterial supply comes over the scala vestibuli and has been described as radiating for some distance in the scala tympani in the basal turn (Ibsen, Charachon, Siebenmann). Nabeya and Smith demonstrated the presence of some venules in the human scala vestibuli, while Siebenmann and Levin found that the frequency of arterioles and venules in this region is similar. The radiating arteriole consists of an endothelial lining with occasional smooth muscle cells and a thin adventitia (Smith, Schucker 1958).

The *capillaries of the apical parts* of the spiral ligament (Fig. 5, 6, 7) form a spiral vessel above the attachment of the vestibular membrane in both the *guinea pig* and *man* (Nabeya, *Smith, Scuderi & del Bo, Perlman & Kimura*, Nomura, Charachon). According to Smith a loose network spreads upward from this vessel to the spiral ligament in man. The capillaries either turn to the collecting venules of the turn immediately above (Smith-guinea pig) or to venules in the scala tympani. The capillaries are composed of endothelial cells and an infrequent smooth muscle cell (Smith).

The *stria vascularis* is situated in the external wall of the scala media (Fig. 5, 6, 7). This is the vascular structure in the cochlea which has excited the most interest due to its apparent role in the formation and absorption of endolymph and as the source of electrical potentials. It also occupies an almost unique position in the literature in having "stria vascularis" as its only name and in being uniformly described by all authors. Retzius (1882) was the first to clearly establish the stria vascularis to be a vascularized epithelium, a question much

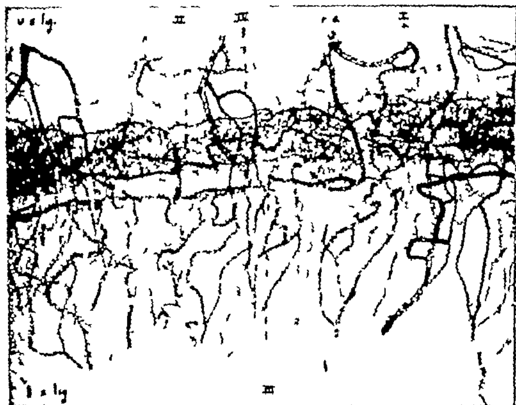


Fig 6 Photomicrograph of a radial section of the external wall in the guinea pig cochlea. l.s.lig. = lower spiral ligament, r.a. = radiating arteriole, u.s.lig. = upper spiral ligament, I = capillary of the upper spiral ligament, II = network of the stria vascularis, III = vessel of the spiral prominence, IV = capillary descending in the depths of the spiral ligament (from Smith, 1951)

debated previously Today there is general agreement that the conception of Retzius was correct. The stria vascularis consists of a capillary mesh-work which is stretched out in a spiral direction and which possesses a rather distinct apical and basal vascular borderline (Nabeya Smith, Charachon) According to Smith and Scuderi & del Bo each radiating arteriole supplies the stria vascularis. The capillaries appear to be composed only of endothelial cells (Smith) Large arterioles enter and large venules leave at regular intervals in man (Smith)

The *vessel of the spiral prominence* was already mentioned by Corti (1851) and by Hensen (1863) In the guinea pig each radiating arteriole supplies a spiral vessel in the spiral prominence (Smith-guinea pig, Nomura) (Fig 6) or a dense network of capillaries in man (Eichler Smith-man) (Fig 5 7) Ramifications from the vessel of the spiral prominence turn to the venules in the scala tympani and to the anastomosing vessels externally to the stria vascularis (Smith)

The *anastomoses in the deeper parts of the spiral ligament* consist of capillaries composed of endothelial cells and infrequent muscle cells in the guinea pig (Smith) They connect the radiating arterioles with the collecting venules (Fig 5 6, 7) In man some are capillaries without perivascular cells others are larger and may represent a kind of arterio-venous shunt (Smith)

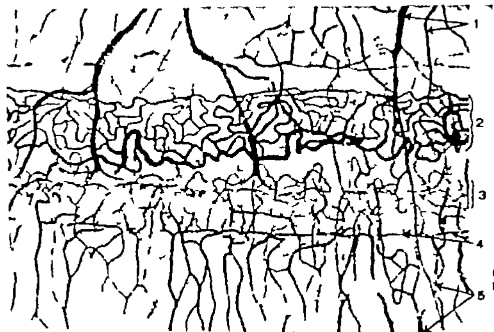


Fig. 7 Photomicrograph of radial section of the external wall in the human cochlea. 1 = radiating arteriol in the scala vestibuli, 2 = stria vascularis, 3 = vessel of the spiral prominence, 4 = spirally running vessel in the spiral crista formed by anastomoses, 5 = collecting venules of the scala tympani (from Scuderi & del Bo, 1952).

The *collecting venules* in the lower part of the spiral ligament drain the different vascular tributaries in the external wall (Fig. 5, 6, 7). The main venous drainage thus goes over the scala tympani. The veins are considered to project a little into the scala vestibuli, but arteries do not enter the scala tympani (Siebenmann, Nabeya). Siebenmann and Charachon demonstrated that arterioles coming from the modiolus supply the scala tympani of the basal turn and parts of the second turn. A spiral vessel has been described in or below the crista ligamenti spiralis (Siebenmann, Nabeya, Smith, Scuderi & del Bo, Svane-Knudsen 1958, Charachon). This vessel marks the upper limit of the vascular network in the scala tympani similar to that found in the scala vestibuli (Smith). Smith considers the spiral vessel in the guinea pig to be a spiral part of the capillaries before they turn to the venules. The venules extend downward over the scala tympani and communicate with the collecting venules. As the collecting venules leave the spiral ligament, a connective tissue adventitia can be demonstrated (Smith).

In vivo studies of the external wall of the guinea pig have confirmed many of these anatomical findings and have contributed knowledge about the circulation in the spiral ligament.

Comment

There are no great differences between the guinea pig and man in the distribution of the vessels in the external wall. Most authors agree that there is an

arterial dominance in the scala vestibuli and a venous dominance in the scala tympani. In fact, many authors totally exclude a venous drainage of the scala vestibuli and an arterial supply to the scala tympani. Spiral capillary vessels have been described as lying above the attachment of the vestibular membrane, in the spiral prominence (a vessel or a vascular network) and in the crista ligamenti spiralis.

Cochlear venous drainage

All the capillary regions described above are drained by venous ramifications collecting to form venules and veins which finally empty into the principal vein of the cochlea the *vein of the cochlear aqueduct* (Fig 1) This vein runs close and parallel to the cochlear aqueduct and empties into the bulb of the jugular vein The anatomy differs in the guinea pig and man.

Guinea pig According to Nabeya, the whole cochlea except a small part at the basal end is drained by the *posterior spiral vein*. This vessel, situated in the lower central aspect of the scala tympani receives venules from the scala tympani on its convex aspect and venules from the spiral lamina, acoustic nerve, spiral ganglion, and the modiolus on its apical aspect. The posterior spiral vein runs a spiral course around the modiolus from apex to base. It empties into the vein of the cochlear aqueduct together with the *vein of the round window* and the *posterior vestibular vein* The vein of the round window drains the region of the round window and the spiral ligament at the basal end. The posterior vestibular vein drains the vestibulum and the most basal parts of the spiral lamina.

Man. The principal vein, the *vein of the cochlear aqueduct* arises when the *anterior and posterior spiral veins* and the two *vestibular veins* join in the basal turn (Eichler Siebenmann Nabeya) There may be additional venous routes formed by connections with vessels in the surrounding bone and with veins in the inner acoustic meatus (Siebenmann) (Fig 2) With regard to man, Nabeya found only occasional connections between the posterior spiral vein and veins in the inner acoustic meatus. The capillaries in the modiolus are drained by branches of the spiral veins

The vein of the spiral lamina (Siebenmann) is situated centrally in the spiral lamina of the basal turn. It runs a spiral course and receives radiating venules from the spiral lamina and the spiral ganglion in the whole cochlea. The vein of the spiral lamina connects the spiral veins and the *internal auditory veins* (Siebenmann) (Fig 2) and is more often found in the basal turn than apically While Eichler failed to demonstrate this vein, Nabeya found it in some cases but not so consistently as Siebenmann Opinions vary concerning the course and distribution of the spiral veins and the internal auditory veins.

Eichler found the principal vein to be the vein of the cochler aqueduct with eight ramifications. The description given of this system is difficult to interpret but probably is essentially correct.

According to Siebenmann there are three principal venous routes from the

cochlea in man. In order of descending importance these are the vein of the cochlear aqueduct, the internal auditory veins, and anastomoses to bone vessels. The posterior spiral vein drains the scala tympani of the basal turn and a small part of the middle turn. In the middle of the basal turn it divides into two branches running in opposite directions, but which subsequently converge. The anterior spiral vein is situated in the scala vestibuli close to the round window. In the modiolus it is situated above the cochlear spiral canal and drains the whole apical region of the cochlea, but only the scala vestibuli and the bone surrounding it in the basal turn. The internal auditory veins have branches which communicate with the posterior spiral vein and the vein of the spiral lamina via the so-called central cochlear vein (Fig. 2). The latter connection has not been confirmed by other authors.

According to Nabeya there are two different systems for cochlear drainage in which either the anterior or posterior spiral vein is significantly larger than the other. In 70 % of the cadavers examined the large posterior spiral vein accounted for more than 2/3 of the blood from the cochlea and the anterior spiral vein the rest.

Comment

The principal venous drainage from the cochlea is carried out by the vein of the cochlear aqueduct. This vein leaves the cochlea at the base in the region of the round window and turns to the bulb of the jugular vein. By the confluence of smaller venules draining the capillary regions, one (guinea pig) or two (man) spiral veins are formed which terminate at the base in the vein of the cochlear aqueduct. There is disagreement about the existence of an additional venous drainage to bone vessels and through the inner acoustic meatus. On the whole it is difficult to form a clear picture of the venous system in the cochlea because of divergent opinions among the various authors.

C. *Measurements of vascular dimensions*

There are only a few measurements of vascular dimensions available in the literature for comparison with those of the present investigation. These are summarized in Table II (page 20). For comments and comparisons see chapter External Wall, Comment.

D. *Summary*

From the published papers it appears that the best technique for the visualization of the blood vessels in the cochlea probably is injection of contrast medium. Studies of the circulation *in vivo* in the external wall give further valuable information on this subject but are, of necessity, limited in scope. Previous descriptions and drawings of the vascular anatomy are often difficult to interpret. This is to be expected since modern stereo- and photo-microscopy were unavailable when these investigations were made. The best available photographic documentation is given by Smith and by Scuderi & del Bo.

TABLE II

Previous measurements of vessel diameters

Author	*Animal*	*Vessel*	*Turn*	*Diameter in microns* *Lumen*	*Vascular wall*
Schwalbe (1887)	guinea pig	Spiral modiolar vein	base	<120	
		Spiral modiolar vein	2nd	60	
		Major arteries in the modiolus	2nd	32 (mean)	
		Radiating arterioles of the "vascular spring-coils"	"mean	10—12	
		Radiating arterioles in the superior part of the spiral ligament		8	
		Radiating arterioles in the scala vestibuli		8—10	
		Collecting venules from the stria vascularis		10	
Nomura (1961)		Radiating arterioles in the external wall	3d, 4th	<20	
Perlman & Kimura (196.)		Spiral ligament vessel		5—8	
		Capillaries of the stria vascularis		8—15	
		Radiating arterioles	"	8—10	
		Collecting venules	"	10	
Perlman, Tsunoo & Spence (1963)		Capillaries of the stria vascularis		8	
		Radiating arterioles		7	
		Arterio-venous anastomoses		5	
Iurato (1962)	rat	Vessel of the basilar membrane		4—7	0.2—1.5
		Vessel of the tympanic lip		4—7	0.2—1.5
		Limbus vessels		3—5	0.2—2
		Small capillaries of the spiral ligament		0.5—2	0.18—1.4
		Larger capillaries of the spiral ligament		≤4	0.18—3
Hinojosa & Rodriguez Echandia (1966)	cat	Capillaries of the stria vascularis			0.5—5
Scuderi & del Bo (1952)	man	Radiating arterioles of the vascular spring-coils		20—40	
Charachon (1961)		Capillaries of the stria vascularis		7	
		Arterio-venous anastomoses		15—20	
		Collecting venules		10—17	
		Capillaries of the scala vestibuli		5—10	
		Radiating arterioles of the spiral lamina	base	30—40	
		Vessel of the basilar membrane		10—20	

The few available descriptions of the vascular anatomy are often conflicting, both concerning major arteries and veins as well as capillary regions. The description of the latter has often been short and incomplete.

The inner ear is supplied by an endartery the labyrinthine artery Before arriving in the cochlea it has divided so that the cochlea is supplied by two arteries, the vestibulo-cochlear artery and the cochlear artery Opinions regarding the existence, course, and ramifications of these vessels vary greatly Contrasting views on the "glomeruli" arcades and a tractus spiralis arteriosus are also numerous.

Previous investigations are more in agreement concerning the capillary regions. In the modiolus these are situated in the spiral ganglion, the acoustic nerve and the modiolus wall. In the spiral lamina there are one or two spiral vessels and a capillary network in the spiral limbus. The vascular arrangement is in general arcadic In the external wall radiating arterioles run over the scala vestibuli and supply the capillary regions in the apical parts of the spiral ligament, the stria vascularis of the scala media, and the vessel of the spiral prominence. Arterio-venous anastomoses externally to the stria vascularis connect the radiating arterioles in the scala vestibuli with the collecting venules in the scala tympani. The latter collect the blood from the above-mentioned capillary regions and empty into one or two spiral veins running around the modiolus. All the cochlear veins confluate to the vein of the cochlear aqueduct. Unlike the arteries, this vein does not leave the cochlea through the inner acoustic meatus but runs parallelly to the cochlear aqueduct and empties into the jugular vein.

III PRESENT INVESTIGATION

A. METHODS

The original aim of the present investigation was to demonstrate the vascular system of the cochlea by a microradioangiographic technique according to Saunders et al (1957) This method, however proved to give too many overlapping vessels in the radiograms. Even if dissected-out pieces of the membranous cochlea were examined, the vascular net was so dense that it proved to be very difficult to discern single vessels and to get a clear view of the vascular anatomy Furthermore, the resolution was insufficient and the transformation of the radiograms to photographs involved a definite loss of information After these disadvantages of microradioangiography were realized, it was decided to attempt the previously used contrast injection method. This method was subsequently modified empirically

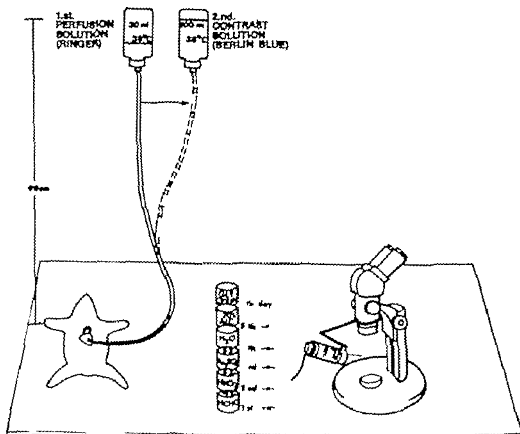


Fig 8 Experimental lay-out illustrating the successive preparative steps in the contrast injection of the guinea pig cochlea

1 *Injection of contrast medium in the guinea pig*

A total of 163 adult guinea pigs, each weighing approximately 300 g, were used in these experiments.

The animals were anesthetized by intraperitoneal administration of urethane, 3 g/kg body weight in a 10 % solution An incision was made in the pericardium and the left ventricle and a plastic tube (Intramedic Polyethylene Tubing PE 160 inner diameter 1 143 mm, outer diameter 1.575 mm, Adams Clay) was inserted in the ascending aorta and ligated. To avoid air emboli the tube was filled with Ringer's solution. The superior vena cava was then cut immediately above the heart and the animal bled for 30 seconds. The animal was subsequently perfused with 30 ml of 38 degree Ringer's solution for 4—7 minutes under a hydrostatic pressure of about 90 cm H_2O (Fig 8) The animal then was injected at the same constant pressure with up to 100 ml of Berlin blue (G T Gurr) at 38 degrees.[1] The vascular system of the animal was perfused until the contrast medium no longer entered it spontaneously Asystole occurred 1—8 minutes after the perfusion was begun. After completed injection, the skull was opened in the occipital region, the temporal bones removed and the tympanic bulla opened.

Preparation of the cochlea

Fixation, decalcification and storage were based upon Schaffer's method (Romeis, 1948)

Fixation	10 % neutralized formalin solution	for 24 hours
Decalcification	5 % nitric acid	" " "
Neutralization	5 % sodium sulfate	" " "
Washing	tap water	" " "
Dehydration	70 % ethyl alcohol	" " "
Storage	water free double-destilled glycerin	

During these preparative steps the entire cochlea, including the windows, was left intact. The cochlea was then ready for visual and photographic examination under the microscope (Fig. 8) The mucoperiosteum was peeled off leaving the cochlea semi-transparent (Fig 9 10) The cochlea then was divided under a dissection microscope in different ways so that all membranous parts could be isolated and photographed. Survey photographs of the complete cochlea were taken by a Hasselblad 500 C camera with a Zeiss S-Planar 5 6/120 mm lens and extension tubes of various lengths. Microphotos were taken of specimens immersed in glycerin between an object slide and a cover glass using a Wild M 20 research microscope provided with phase-contrast equipment and a camera with Kodak Tri-X Pan film, 27 DIN Phase-contrast microscopy was used in order to make uninjected vessels visible

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[1]) The contrast medium was prepared from a Berlin blue powder (soluble Prussian blue, ferric ferrocyanide) dissolved in Ringer' solution for 8 hours at 40 degrees to a concentration of 2.5 It was then filtered twice at 40 degrees through a Pyrex filter pore size 1 (100—120 microns) with negative pressure of 2.5 kg. The particle size of the final injection solution was less than 0.5 microns and the pH 5.75.

Comment

The method described is the result of modifications of previously published techniques. The modifications were introduced to refine the contrast injection technique and to shorten the process of preparation.

A number of steps have been varied within the following limits without any significant influence on the result of the injections.

a. *Quantity of the preceding perfusion.* 30—200 ml of Ringer's solution or no perfusion at all gave the same result.

b. *Quantity and concentration of the Berlin blue solution.* 15—100 ml of 2—10 % Berlin blue dissolved in Ringer's solution.

c. *The injection pressure.* Hydrostatic within the limits 50—120 cm H_2O. It proved very difficult to find a pressure which allowed the filling of all vessels in the cochlea without rupturing the vessels in the inner meatus.

d. *Decalcification agent.* 5 % nitric acid, 5 % hydrochloric acid, 10 % formic acid, EDTA, Parengy, 5 % trichloroacetic acid.

e. *Degree of dehydration.* The complete series of dehydration steps to 96 % ethyl alcohol was not found to be necessary. A satisfactory dehydration was achieved with a 24 hour immersion in 70 % ethyl alcohol.



Fig. 9. [illegible] demonstrating the relatively accessible cochl[illegible]



Fig. 10 Guinea pig cochlea. Left: the dense vascular net of the periosteum exposed. Arrow = main artery of the vascular net. Right: removal of the periosteum leaves the cochlea semi-transparent. Radiating arterioles predominate in the scala vestibuli and collecting venules in the scala tympani. VRW = vein of the round window. SVS = stria vascularis (17X).

Variation of the following steps appeared to significantly alter the result of the injection:

a. *The stage of anesthesia.* When the animal still reacted to pain the subsequent injection was less successful than if the animal was in deep general anesthesia.

b. *Bleeding of the animal before injection.* Better results were obtained when the animal was bled, regardless of where it was bled (section of the superior vena cava, incision in the liver, cardiotomy in the right ventricle).

c. *Nature of the contrast material.* Berlin blue, Indian ink, and Evan's blue were used. Berlin blue gave a markedly better and more uniform contrast injection than the others.

d. *The storage medium.* Immersion oil, Epon and glycerin were used. Storage in glycerin, used previously only by Konaschko (1927), proved to have many advantages. It is a transparent and nonvolatile medium. The latter property is important since the danger of evaporation is particularly great during the examination of the specimen under the strong microscope illumination. The cochlea keeps its elasticity in glycerin after decalcification. The color of the contrast did not change even after three years storage of both whole cochleas and dissected-out pieces. Further dissection can easily be carried out if desired. Whole cochleas and dissected-out pieces can be photographed in glycerin.

Subsequent examination with phase-contrast microscopy demonstrated that the capillaries of almost all areas of the cochlea were filled with contrast in the well injected preparations. A common exception was the stria vascularis where small parts of the capillary net often were uninjected. This could also be observed without phase-contrast as the vessels generally were injected to at least some extent, revealing their position. Ruptured vessels in the cochlea or coloration of the intralabyrinthine fluids were never observed.

2 *Injection of contrast medium in man.*

For these experiments 60 cadavers of different ages were used Individuals with presumed cerebral or auditory circulation disturbances were excluded by the case histories.

The injection procedure was principally similar to that employed with the guinea pig cochlea. The cadaver was injected as soon as possible after death, preferably within 24 hours The vertebral arteries were dissected free and cut at their departure. In some cases the brain was removed A plastic tube (see specifications for the guinea pig) was inserted into the basilar artery from its anterior end and installed with its tip in the region of the departure of the anterior inferior cerebellar artery In those cases where the brain was not removed, the plastic tube was inserted into one or both vertebral arteries. Perfusion was achieved with 400 ml of Ringer s solution at a hydrostatic pressure of 120 cm H_2O Subsequent contrast injection was carried out with 400 ml of 3 % Berlin blue solution at the same hydrostatic pressure. In the beginning of the perfusion and contrast injection, free flow was allowed, but soon intermittent occlusion on the arterial system was introduced to direct the flow As only minimal back flow could be detected through the venous system of the neck, no veins were occluded. Immediately after completed injection the temporal bones were removed and put in fixing solution

Preparation of the cochlea

The temporal bones were fixed in 10 % formalin for 48 hours The subsequent preparative steps were the same as presented for the guinea pig with the exception that the decalcification procedure required several days, during which a successive dissection of the cochlea was performed. Intact and dissected cochleas were studied under the dissection-microscope and photographed in the same way as described for the guinea pig

Comment

As with the guinea pig it was not possible to achieve uniformly good results with the method presented in spite of many variations in the injection technique.

The following variations did not appear to influence the results

a *Age and cause of death*

b *Temperature of the perfusion solutions* 20 or 37 degrees

c *Temperature and concentration of the Berlin blue solution.* 20 or 37 degrees, 2.5—6 %, diluted with Ringer s solution or 6 % Macrodex filtered twice at 40 degrees in the same way as described for the guinea pig.

d *Presence or absence of the brain*

e. *Removal of clots in the venous sinusoids in the skull*

f *Site of injection* Basilar vertebral basilar + vertebral arteries.

g *Stasis* Regulation of the flow of perfusion and contrast solutions into the venous system.

The following factors seemed to influence the results:

a. *Interval death — injection.* If this interval was short the results usually were better.

b. *Anemia or hemorrhage.* These conditions in connection with death seemed to have a favorable influence on the injection.

c. *Quantity of the perfusion and contrast solution.* Large volumes led to better results.

d. *Injection via the jugular vein.* This was not successful as the contrast medium flowed directly into the venous sinusoid system.

e. *Injection pressure.* Besides hydrostatic pressure, manual injection by syringe and controlled air pressure were attempted. The latter technique involved starting with low hydrostatic pressure which subsequently was raised to over 300 mm Hg by application of air pressure to the reservoir of contrast medium. This method gave satisfactory results in many cases. As in the guinea pig it is difficult to adjust the pressure of injection so that the contrast passes the vessels of the inner acoustic meatus without rupturing them.

Occasionally simultaneous injections in the vertebral and internal carotid arteries were attempted. This method of injection seemed promising, but it had to be abandoned because of disfigurement of the face of the cadaver.

It was not possible to judge if the injection had been successful while the procedure was still being carried out. In most cases all superficial vessels of the cerebellum and the basal parts of the cerebrum were well filled. There was, however, no correlation between the degree of filling of these vessels and those of the cochlea.

Often basal parts of the cochlea were well filled with contrast while apical parts were not. In other cases the opposite condition was found. No extravasates occurred around the cochlear vessels, nor was coloration of the intralabyrinthine fluids found. In all cases the vessels of the inner acoustic meatus were injected and the surrounding tissues and meninges as well. In many instances there had been ruptures in the inner acoustic meatus and resulting completely uninjected vascular areas in the cochlea. Differences in contrast filling between the arterial and venous systems were striking in some cases. The stria vascularis and the capillary regions in the spiral lamina proved particularly difficult to fill completely. This also applied to the stria vascularis even if other parts of the external wall were well injected. This effect appears to be due to a shunting over of contrast along the "path of least resistance" directly from the arterial system on the scala vestibuli to the venous system on the scala tympani.

The dissection of the human cochlea is much more difficult than that of the guinea pig. The position deep in the temporal bone, the difficulty of removing this bone, the great diameter of the basal turn which makes observations from the outside difficult, and the strong attachment of the basilar membrane to the external wall contribute to these difficulties. Furthermore, the guinea pig can be injected *in vivo*, thus avoiding the clots which obstruct the vascular system of human cadavers.

3 *Measurements of the vascular anatomy in the guinea pig*[1]

The animals were injected with contrast according to the method described Whole cochleas, radial and transverse sectioned cochleas, and small dissected-out pieces from the membranous cochlea were used for the measurements. The following parameters were investigated under a stereo-microscope

a. *Number of radiating arterioles in the scala vestibuli*

b *Distribution of the radiating arterioles to different vessels in the external wall*

c. *Occurrence of vascular anastomoses in the external wall and the spiral lamina*

The results of *a* and *b* are presented as means of measurements in 10 animals.

In addition photomicrographs were taken of glycerin-embedded preparations. The magnification used was 7X—375X. The material was composed of samples from 7 guinea pigs A measuring ocular (graduated in 0 1 mm) and an odometer (graduated in cm) were used for making the following measurements:

a *Distances between vessels*

b *Diameter of vessels*

c. *Vascular density* (vessel length/tissue area)

The material in the latter three measurements was treated statistically according to the methods of two-way analysis of variance and Tukey s procedure was adopted for the calculation of individual differences between turns (Brownlee, 1961) The total variance was decomposed into the following categories

variance depending upon differences between animal means.

" " " " " turn means.

" " " interactions.

" " " differences between observations per animal and turn.

Level of significance· 5 %

Number of animals 7

Number of turns: 3

Number of photos per turn 2 (5 measurements per photo)

Comment

Studies hitherto available have furnished valuable information concerning the descriptive vascular anatomy and observations *in vivo* in the external wall There is, however a lack of published data concerning vascular diameters, ramifications, density etc. The present method does not pretend to reflect *in vivo* conditions concerning vessel diameters. Although the aim of the technique was to measure the diameter of the vessel lumen a number of experimental variables can influence this value:

a Possible staining of the vascular wall by the injected medium, thus causing the wall thickness to be included in th measurement.

[1] These examinations were carried ou together with Berthelm Maas, M.D Department of Otolaryngology U iversity of Dusseldorf

b. Distension of the vessel as a function of the injection pressure and the possible presence of some smooth muscle activity in the vascular walls.

c. Distortion of the tissues during the different steps of post mortem preparation.

The precise degree to which these different factors contribute to the measured diameter is difficult to evaluate. However if the vascular walls were stained, one would expect that *the contrast medium would pass the endothelial cells* and stain the surrounding tissues as well, which was never the case. Further arterioles in which the smooth muscles are completely relaxed, which probably is the case during the preparation, are no doubt to some extent distensible (Folkow & Löfving 1956). The diameter changes are of relatively moderate degree, possibly within 20—30 %, when the transmural pressure is reduced from a normal level to the low pressure level which exists at the moment the tissues are fixed. Capillaries in general seem to be very little distensible. Veins do not demonstrate any high degree of true distensibility but rather a collapse of their lumen at very low distending pressure (Öberg, 1967). Further it is unlikely that vascular smooth muscle activity should remain to any significant extent since the animal is exposed to both hypoxia and the low pH of the injected medium during the experimental procedure.

In any case, the present method facilitates the visualization of the vascular arrangement, the calculation of the number of vessels, the measurement of distances between vessels, the estimation of the course, ramification and termination of the vessels and approximate estimation of vascular dimensions. The results thus provide new data.

4. *Nomenclature*

The anatomical nomenclature adopted in the present investigation is given in Fig. 11. The vascular nomenclature has in general followed the available one

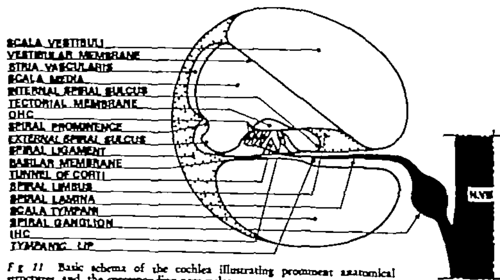


Fig. 11. Basic schema of the cochlea illustrating prominent anatomical structures and the corresponding nomenclature.

concerning artery — arteriole — capillary — venule — vein However a strict anatomical nomenclature based only on the criterion of vessel diameter has been abandoned and the commonly used nomenclature preferred. This was necessary since it was not the aim of the present investigation to carry out histological studies of the vascular wall By arterio-venous anastomosis is here meant the shortest vascular connection between the arterial and venous systems with a rapid blood flow It may or may not have the function of a capillary If trans-mural exchange occurs, which can only be settled by functional studies, the term "arterio-venous thoroughfare capillary" would be most appropriate. If no exchange is possible and therefore true shunting of blood occurs, the term "arterio-venous anastomosis" or arterio-venous shunt" would be most suitable (Illig, 1961)

Identification of individual vessels was accomplished by following them from their origin in the readily identifiable major arteries and veins. Descriptive names have been suggested for certain vessels which hitherto have not been named in order to simplify future discussion Suggestions have also been made to alter the established nomenclature of some vessels. In these cases, justification has been provided for the new name.

The different structures of the cochlea have been referred to in the following way

Apical — basal any axis parallel to the midmodiolar axis.

Central — peripheral any axis perpendicular to the midmodiolar axis.

Radial section section parallel to the midmodiolar axis.

Transverse section: Section perpendicular to the midmodiolar axis.

Above — below apical and basal, respectively to a given point or structure used in describing localized areas of the cochlea.

Spiral the helical course around the midmodiolar axis described by the cochlea.

Turn a 360 degree segment of the cochlear spiral.

Modiolus the bony central axis including the acoustic nerve, spiral ganglion and vessels.

Spiral lamina the spiral plate consisting of the osseous and membranous spiral lamina as well as the basilar membrane

External wall the peripheral tissue layer including the spiral ligament covering the cochlea from the most apical point of the scala vestibuli to the most basal point of the scala tympani.

Basal end: the partially uncoiled most basal segment of the cochlea.

5 *Summary*

The following method has been elaborated with the aim of visualizing the vascular anatomy of the cochlea in the guinea pig and man injection of contrast medium decalcification, dissection under the stereo-microscope, and photo-microscopy With this method the vascular anatomy of the cochlea has been described and measurements carried out.

B. RESULTS

1 *The vascular anatomy of the modiolus*

a. *Guinea pig*

Arterial system

The *spiral modiolar artery* (SMA, A. spiralis modioli) is the direct continuation of the labyrinthine artery and its terminal branch (Fig. 12, 13) From its entrance in the modiolus, SMA is always situated in the interspace between the acoustic nerve centrally and the spiral ganglion peripherally The artery is situated basally in the interspace and its branches more apically (Fig 12, 13 14) SMA runs an irregular serpentine course particularly at the base. The size of SMA gradually diminishes (Fig 21 Table III) and in the apical turn the single arterial stem has divided into 2—4 terminal branches.

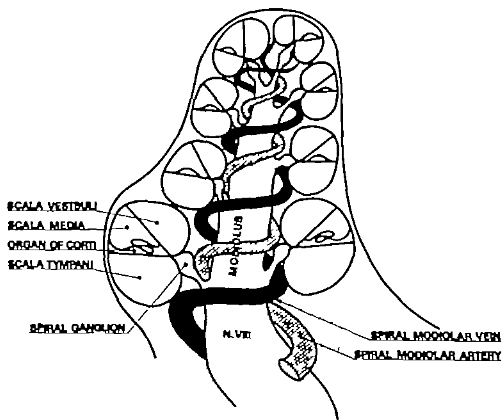


Fig 12. Guinea pig cochlea, radial section, schematic. The main artery and vein in relation to the modiolus and scalas.

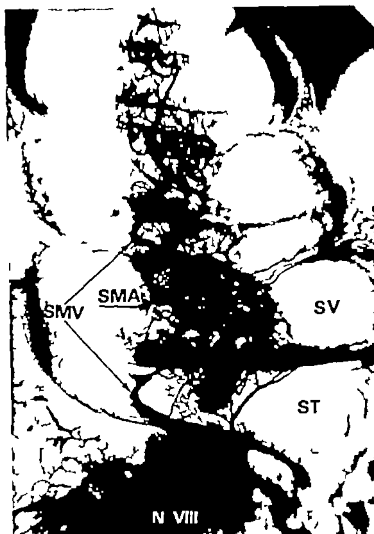


Fig 13 G mea pig cochlea, radial section. Spiral modiolar artery (SMA) with primary and secondary rterial branches run ning around the modiolus at the level of the scala vestibuli with a winding course. Spiral modiola vein (SMV) running around the modiol in the central-basal aspect of the scala tympani with straighter course SV = scala vestibuli, ST = scala tympani, N VIII = acoustic nerve arrow = capillary net the modiolus w ll (32.5X).

Two kinds of branches are given off Large primary branches are not numerous but are given off regularly usually two in each turn (Fig. 13 14) They have a serpentine course and sometimes make loops. The primary bran ches soon divide into several secondary and further ramifications with an increasingly winding course resembling spring-coils. These structures were previously termed glomeruli. The radiating arterioles run from the modiolus to apical parts of the scala vestibuli and to the spiral lamina and limbus (Fig. 14 15 19) Centrally in the spiral lamina the arterioles sometimes connect with one another to form spirally running arcades (Fig 16) The diameter of the

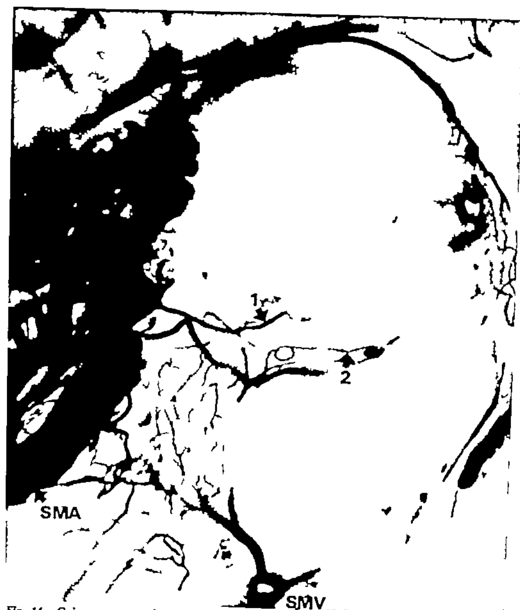


Fig 14 Guinea pig, second turn, radial section. Spiral modiolar artery (SMA) with winding course. Primary and secondary branches forming frequent loops. Spiral modiolar vein (SMV) receiving branches from the external wall (right) and the spiral lamina and ganglion (left). Arrow[1] = arteriole supplying the spiral limbus, arrow[2] = arterioles supplying the marginal vessels f the spiral lamina (75X).

radiating arterioles in the modiolus is somewhat greater in the basal than in the second and third turns (Fig. 21 Table III) SMA also gives off ramifications of quite different appearance. They are smaller and straighter and take part in the supply to the wall of the modiolus, the acoustic nerve, and the spiral ganglion (Fig 16) They are numerous basally and form a vascular net around the artery

Fig 15 Guinea pig, modiolus, transverse section. Spiral modiolar artery giving off primary and further branches with an increasingly winding course forming "vascular spring-coils" (195X).





Fig 16 Guinea pig, modiolus, transverse section. N VIII = acoustic nerve surrounded by spiral modiolar artery. A dense net of capillaries surrounding the spiral modiolar artery is revealed upon further dissection (right). LVS = limbus vessels, arrow = arcade between radiating arterioles (68X).



Fig. 17. Guinea pig, radial section. VCAQ = vein of the cochlear aqueduct leaving the cochlea parallel to the cochlear aqueduct and not by way of the inner acoustic meatus. SMV = spiral modiolar vein, SMA = spiral modiolar artery. N VIII = acoustic nerve, arrow = vascular net on the modiolus wall (22X).



Fig. 18. Guinea pig, modiolus, basal turn, transverse section. Spiral modiolar vein running around the modiolus receiving several collecting venules of the scala tympani on convex aspect and a few from the acoustic nerve, spiral ganglion and the spiral lamina on apical or medial aspect. Arrow = venule penetrating nerve radially (41X).

Venous system.

The *spiral modiolar vein* (SMV V. spiralis modioli) originates in the fourth turn as the confluence of the venules from the capillary regions in the apical turn. SMV runs a spiral course around the modiolus in the whole cochlea (Fig. 12 13). It is always situated in the basal central angle of the scala tympani (Fig. 14). As compared with SMA the vein has a more even course and its diameter gradually increases toward the base (Fig. 21. Table III). At the base close to the round window SMV curves about 90 degrees and empties into the vein of the cochlear aqueduct (Fig. 17). SMV drains most of the capillary regions of the cochlea. Numerous primary branches coming from the external wall of the scala tympani empty into its convex aspect (Fig. 14, 18). The diameter of these

Fig 19 Guinea pig, modiolus, second turn, transverse section. Central-left: acoustic nerve with capillaries. Central-right: spiral ganglion. Above: spiral modiolar vein. Below-left: "vascular spring-coils" of the radiating arterioles. Below right: vessels in the spiral lamina and limbus (95X).



Fig 20 Guinea pig, modiolus, second turn, transverse section. Spiral ganglion with arterial supply centrally and venous drainage forming margin peripherally. SMA = spiral modiolar artery (144X)

TABLE III

The diameter of the vessels in the guinea pig modiolus

Mean diameter of:	Turn Base	2nd	3rd	SD[1]	Significant Differences
spiral modiolar artery[2]	82.5	70.9	47.8	17.3	AI $T_1>T>T$
spiral modiolar vein	84.9	64.0	44.8	14.5	AI $T>T>T$
radiating arterioles[3]	15.0	12.0	12.0	3.2	AI $T>T=T$
collecting venules[4]	25.3	14.1	13.4	9.7	$T>T=T$
capillaries in acoustic nerve	5.4	4.7	4.5	2.5	A $T=T=T$

All measurements in microns.
[1]) SD = standard deviation (error)
[2]) Measured in 6 animals due to insufficient material.
[3]) Measured immediately peripherally to the "vascular spring-coils"
[4]) Measured immediately before emptying into the spiral modiolar vein.
A = statistically significant difference between animals.
I = statistically significant interactions.
T = statistically significant difference between turns in the same animal.
(T_1 = basal turn, T_2 = second turn, T_3 = third turn)

venules is much greater in the base than in the second and third turns (Fig. 21, Table III). A small number of primary branches deriving from the capillary regions in the spiral lamina, spiral limbus, spiral ganglion, acoustic nerve and the wall of the modiolus are received by its apical aspect (Fig. 13, 14, 17, 18). In the apical turn there is occasionally a special arrangement in which venules deriving from the scala tympani merge with venules from the modiolus wall before emptying into SMV. Basally in the cochlea these branches turn to SMV separately.

The basal end of the cochlea is drained by the vestibulo-cochlear vein and its branch, the vein of the round window. The vein of the cochlear aqueduct is formed by the confluence of SMV and the vestibulo-cochlear vein. The anatomy of these veins is described in the chapter Basal End.

Capillary system

The capillary regions of the modiolus are situated in the acoustic nerve, in the spiral ganglion, and in the wall of the modiolus.

The *acoustic nerve* is penetrated by a net of capillaries. Particularly at the base, larger arterial and venous branches also penetrate the nerve (Fig. 18). In general the capillaries have a spiral course peripherally and a straighter apico-basal course centrally without any regular pattern (Fig. 16, 19). The diameter

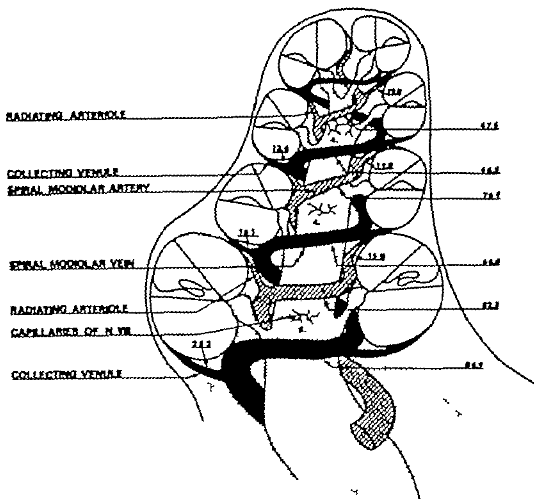


Fig 21 Guinea pig, radial section, schematic. Measurements in microns.

of the capillaries is more or less the same in the three basal turns, i.e. about 5 microns (Fig. 21 Table III)

In the *spiral ganglion* the arterial supply derives centrally and the venous drainage peripherally (Fig. 14 19 20) Small venous branches often make up a margin of arcades. The capillaries in the spiral ganglion otherwise do not show any characteristic arrangement

In the *wall of the modiolus* there is also an irregular vascular net. Most of the vessels are arterioles and venules running to the capillary regions in the scala vestibuli, in the spiral lamina and in the scala tympani From these arterioles and venules ramifications are given off which contribute to the vascular net on the modiolus wall (Fig. 13 17)

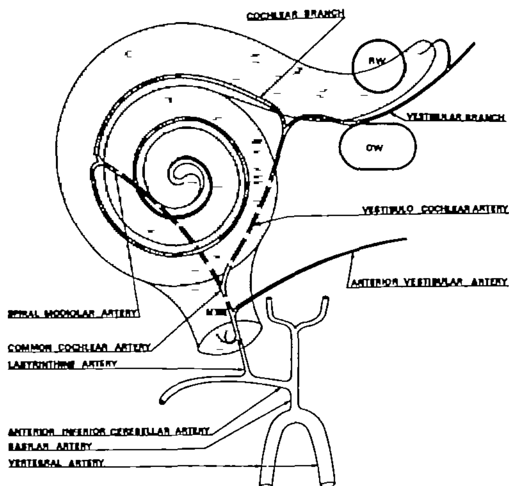


Fig. 22. Man, arterial system, schematic. OW = oval window RW = round window N VIII = acoustic nerve.

b. *Man*

Arterial system

After the ramification of the anterior vestibular artery the labyrinthine artery is called the common cochlear artery (Fig. 22) It soon divides into two terminal arteries, the vestibulo-cochlear artery and the spiral modiolar artery the latter formerly called the cochlear artery (A. cochleae propria)

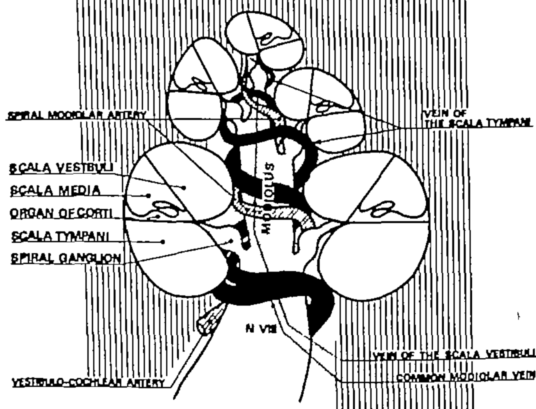


Fig 23 Man, modiolus, radial section, schematic. The basal half of the basal turn is supplied by the vestibulo-cochlear artery; the rest of the cochlea by the spiral modiolar artery In contrast with the guinea pig, there is a double enous drainage, one in the scala vestibul and one in the scal tympani.

In the inner acoustic meatus the *vestibulo-cochlear artery* (VCA A. vestibulo-cochlearis) is hidden by the coiling of the cochlear nerve, but can easily be observed if the latter is extended. On its course to the modiolus branches are given off to the surrounding tissues. Arriving in the modiolus it divides into two terminal branches, the vestibular and cochlear branches (Fig 22, 23 24).

The *vestibular branch* (VB Ramus vestibularis) supplies the basal end of the cochlea and the vestibulum. It is further described in the chapter Basal End.

The *cochlear branch* (CB Ramus cochlearis) runs in the opposite direction to VB i.e apically It is situated above the spiral ganglion and runs a serpentine course. It supplies 1/4 to 1/2 of the basal turn and ends by anastomosing with one or two basally running branches from the spiral modiolar artery (Fig 22, 24 25 26) As the two branches anastomose it is not possible to judge the exact

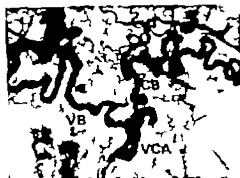


Fig. 24 Man, modiolus, basal turn, radial section The vestibulo-cochlea artery (VCA) ramifying to form two terminal branches the cochlear branch (CB) and the vestibular branch (VB). Note the serpentine winding course (27X)

Fig. 25 Man, basal turn, transverse section. AVA = anterior vestibular artery AVV = anterior vestibular vein, CB = cochlear branch of the vestibulo-cochlear artery with winding course, giving off radiating arterioles (RAL) over the scala vestibuli. Arrow = region where the spiral modiolar artery arrives in the cochlea. VSV = vein of the scala vestibuli, SVS = stria vascularis at the basal end, forming one or two large vessels (12X)

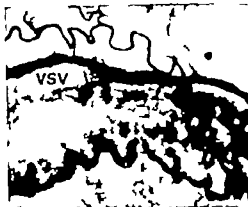


Fig 26 Man, modiolus, basal turn, transverse section. Double anastomosing vessels between the cochlear branch of the vestibulo-cochlear artery and the spiral modiolar artery Not the serpentine course. VSV = vein of the scala vestibuli (27X).

Fig 27 Man, basal turn, radial section SMA = spiral modiolar artery VSV = vein of the scala vestibuli, VST = ein of h scal tympani draining the spiral ganglion and external wall (30X).



Fig 28 Man, basal-second turn, transverse section, dimected. Venous system, VSV = vein f the scala vestibuli, VST = ein of the scala tympani, CMV = common modiolar vein, AVV = anterior vestibular vein (the posterior is uninjected), VCV = vestibulo-cochlear vein, VCAQ = vein of the cochlear aqueduct (7X).

region supplied by a given branch. From CB arterioles are given off to the capillary regions in the modiolus, the spiral lamina, and the external wall.

In the inner acoustic meatus the *spiral modiolar artery* (SMA, A. spiralis modioli) is even more hidden by the coiling of the acoustic nerve than the VCA. SMA arrives in the modiolus about a half turn from the basal end of the cochlea (Fig 22, 23 25) The main stem follows the spiral course of the cochlea up to the apex and supplies the whole cochlea except the first basal half turn. The position of SMA is above the spiral ganglion in the modiolus (Fig 27) The first branch running basally is described above. SMA has a pronounced serpentine course, particularly apparent in the base and straightening somewhat toward the apex. From the main stem radiating arterioles are given off at right angles, supplying the capillary regions in the modiolus, in the spiral lamina, and in the external wall. At their departure the radiating arterioles have a distinctly serpentine course and may also make loops but do not appear as the "vascular spring-coils" found in the guinea pig Centrally in the scala vestibuli they connect with one another forming spirally running arcades.

The present investigation confirmed the findings of Nabeya and Charachon that SMA in certain individuals is replaced by the cochlear branch of the vestibulo-cochlear artery This branch then continues to the apex, supplying the capillary regions otherwise provided for by SMA.

Venous system

The venous system is more complicated in man than in the guinea pig. In the whole cochlea there are thus separate venous systems in the scala vestibuli and in the scala tympani with corresponding veins running spirally around the modiolus (Fig 23 29)

There is a spiral vein in the basal central angle of the scala tympani corresponding to the spiral modiolar vein in the guinea pig This is called the *vein of the scala tympani* (VST V scalae tympani) It is not, however continuous

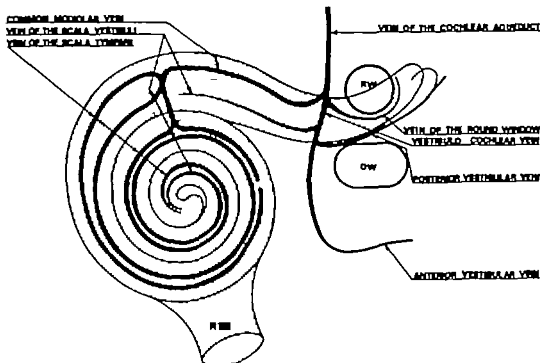


Fig 29 Man, venous system, schematic. Contrary to the arterial supply and to the case in the guinea pig, there is a venous drainage of both the scala vestibuli and the scala tympani. There are great variations in the course and distribution of enous ramifications. OW = oval window RW = round window N VIII = acoustic nerve.

from the apex to the base as in the guinea pig, but is rather formed by a series of venous segments often running in opposite directions. This is most easily seen in Fig 29 The veins which make up VST drain the spiral ganglion and the external wall of the scala media and of the scala tympani (Fig 27 28) Occasional ramifications run toward the tractus spiralis foraminosus. It was not possible to establish whether they connect with veins in the surrounding bone or with veins of the inner acoustic meatus.

The *vein of the scala vestibuli* (VSV V scalae vestibuli) is also composed of separate venous segments and drains the spiral lamina and the scala vestibuli of the whole cochlea (Fig 23 25 26 27 28) The first 1/4 turn is an exception in that it is drained instead by the vein of the round window and the posterior vestibular vein. VSV is situated centrally in the scala vestibuli in the modiolus wall The complicated course of its different ramifications is most easily demonstrated in Fig 29 No two human cochleas were observed to be identical with respect to variations in the course distribution and frequency of connections between the vein of the scala tympani and VSV At varying distances from the basal end, usually about 1/3—1/4 turn, the veins forming the VST and VSV join to form the *common modiolar vein* (CMV V modioli communis) (Fig 23 28 29) CMV runs toward the basal end of the cochlea in the central basal angle of the scala tympani Just before arriving at the round window it turns sharply confluates with the vestibulo-cochlear vein and thus forms the vein of the cochlear aqueduct. The latter two veins are described in the chapter Basal End.



Fig 30 Man, basal turn, radial section. Spiral ganglion with irregular capillaries. Arterial supply above and venous drainage below (155 X).

Capillary system

The capillary regions are situated in the acoustic nerve, in the spiral ganglion, and in the modiolus wall.

In the *acoustic nerve* there is a sparse net of fine capillaries which are often difficult to inject completely with contrast medium. They are supplied from the vestibulo-cochlear artery and the spiral modiolar artery and are drained by both the vein of the scala vestibuli and the vein of the scala tympani. The capillaries have an apico-basal course without any regular pattern

The very numerous capillaries in the *spiral ganglion* make it one of the most vascularized areas in man. They do not, however demonstrate any typical arrangement (Fig. 30) The spiral ganglion is supplied by arterial branches centrally and venous branches peripherally as in the guinea pig. In the *modiolus wall* there is a net of arterioles, venules and capillaries whereas venules dominate in the scala tympani. The vessels are not so prominent as in the guinea pig and apparently lack a fixed system.

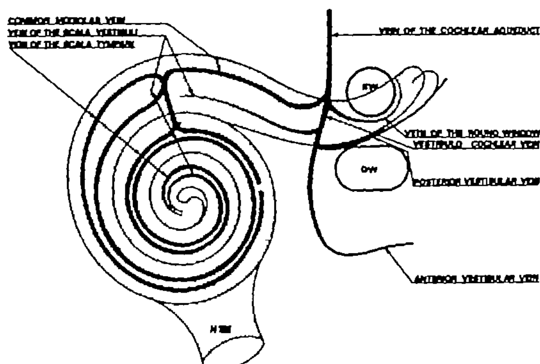


Fig. 29. Man, venous system, schematic. Contrary to the arterial supply and to the case in the guinea pig, there is a venous drainage of both the scala vestibuli and the scala tympani. There are great variations in the course and distribution of venous ramifications. OW = oval window RW = round window N VIII = acoustic nerve.

from the apex to the base as in the guinea pig but is rather formed by a series of venous segments often running in opposite directions. This is most easily seen in Fig. 29 The veins which make up VST drain the spiral ganglion and the external wall of the scala media and of the scala tympani (Fig 27 28) Occasional ramifications run toward the tractus spiralis foraminosus. It was not possible to establish whether they connect with veins in the surrounding bone or with veins of the inner acoustic meatus.

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2. *The vascular anatomy of the spiral lamina and the spiral limbus*

a. *Guinea pig*

The following vascular structures can be identified in the *spiral lamina*

Radiating arterioles
Collecting venules
The vessel of the basilar membrane }
The vessel of the tympanic lip } Marginal vessels

and the following in the *spiral limbus*

Radiating arterioles
Collecting venules
The limbus vessels

The *radiating arterioles* (RAL, Arteriolae radiate) are tertiary or further ramifications of the spiral modiolar artery (Fig 31) and are situated more basally in the modiolus than the vessels supplying the external wall. Peripherally to the "vascular spring-coils" in the modiolus the RAL run through the bony parts of the modiolus to the osseous spiral lamina (Fig 13 14) They continue

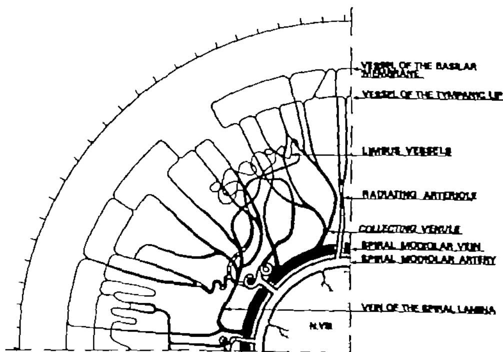


Fig 31 Guinea pig and man, spiral lamina and limbus, schematic. The vascular system is arcadic with peripherally radiating arterioles, spirally running capillary vessels, and centrally radiating venules. The region peripheral to the vessel of the basilar membrane is principally avascular. The vessel of the basilar membrane is situated under the tunnel of Corti. The vessel of the tympanic lip, which is located in the region of the habenula perforata, receives most of the radiating vessels. Capillaries in the spiral limbus form irregular limbus vessels. N VIII = acoustic nerve.

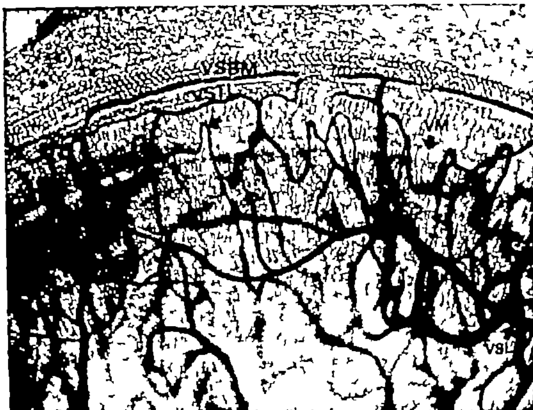


Fig 32. Guinea pig, spiral lamina, basal half f the basal turn, transverse section, phase contrast VSBM = the vessel of the basilar membrane which is located centrally to the outer hair cells and below the tunnel of Corti and is almost continuous. VSTL = the vessel of the tympanic lip which is situated below the habenula perforata and is more uneven and discontinuous. Arrow = arcades centrally to VSTL, VM = attachment of the vestibular membrane, VSL = vein of the spiral lamina recei ing collecting venules (155X).

radially in the tympanic lip and the basilar membrane, supplying the marginal vessels. The branches connecting with the vessel of the basilar membrane are often of greater diameter than those for the vessel of the tympanic lip and the limbus vessels (Fig 32, 33) Each RAL supplies a rather small segment. The diameter of the capillary ramifications of the RAL and the collecting venules immediately central to the vessel of the tympanic lip is less than that of the marginal vessels (Fig. 33 37 Table IV) The angles at the ramifications of the RAL and collecting venules are approximately 60 degrees. When the RAL reach the marginal vessels, they turn off squarely in a T formed manner or build up arcades (Fig 32) The branches from the RAL and collecting venules supply the vessel of the tympanic lip with about three times as many vessels as the vessel of the basilar membrane receives (Fig 37 Table IV) This condition is maintained in the three basal turns. The finest capillary ramifications of the RAL take part in the formation of an irregular sparse capillary network central to the marginal vessels.







Fig. 33. Guinea pig, spiral lamina, second-third turn, transverse section, phase contrast. Examples of exceptions to the straight continuous appearance of the marginal vessels. Above: umbrella-like structures of the vessel of the basilar membrane. Middle: double vessel of the basilar membrane and the vessel of the tympanic lip. "Umbrella". Below: double vessel of the basilar membrane and the vessel of the tympanic lip. The latter is partially replaced by arcades (left). OHC = outer hair cells, arrow = attachment of the vestibular membrane (375X)

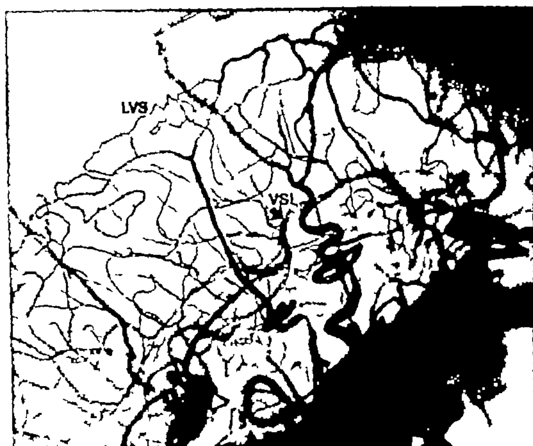


Fig 34 Guinea pig, spiral limbus, basal-second turn, transverse section. Radiating arterioles with vascular spring-coils supplying the spiral limbus. An irregular peripheral margin, the limbus vessels (LVS), is formed by the arcades. Collecting venules drain the limbus vessels, often terminating in the vein of the spiral lamina (VSL). Note the capillary net centrally in the spiral limbus (95X).

Peripherally in the spiral lamina there are two parallel, spiral vessels which run at right angles to the radiating vessels. They constitute a vascular arcade and make up two vascular margins. The marginal vessel lying most peripherally is the *vessel of the basilar membranae* (VSBM, Vas membranae basilaris) (Fig 31 32 33) VSBM is situated below the tunnel of Corti and can be demonstrated in the whole cochlea between the basal and apical ends of the spiral lamina. Principally VSBM curves smoothly following the organ of Corti throughout the whole cochlea. In the first half of the basal turn it is completely continuous, but apically one may find an increasing number of interruptions as well as a more uneven course (Fig. 32, 36) Occasionally the stems of the supplying vessels protrude peripherally beyond the level of VSBM forming umbrella-like structures with the two T arms curving back centrally to the vessel which can also be double for short distances (Fig 33) The deviation from the right angle is generally within ?0 degrees at the joining of the radiating vessels and VSBM



Fig 35 Guinea pig, spiral limbus, basal turn, transverse section, phase contrast. Peripherally the limbus vessels form an irregular margin. Note open sparse loops of the capillaries centrally Arrow = Huschke dents, i e. limbus cells (184X).

Peripherally in the tympanic lip and below the habenula perforata, parallelly and centrally to VSBM lies the other marginal vessel the *vessel of the tympanic lip* (VSTL, Vas labii tympanici) (Fig 31 32, 33) The supplying and draining radiating vessels meet VSTL within 15 degrees of the right angle. In the first quarter of the basal turn VSTL is replaced by capillary arcades which lack a spiral marginal appearance. In the second quarter these arcades and VSTL can be demonstrated simultaneously (Fig 32) From the second half of the basal turn VSTL can be demonstrated to the apex. Apically it differs from the vessel of the basilar membrane in having more frequent interruptions and in running more unevenly Particularly in the apical turn, short radiating connections from VSTL regularly communicate with the vessel of the basilar membrane. The diameter of the two marginal vessels is about 5 microns with VSTL usually being the larger of the two. The diameter is well maintained in the three basal turns for both marginal vessels (Fig 37 Table IV) The region between the two mar

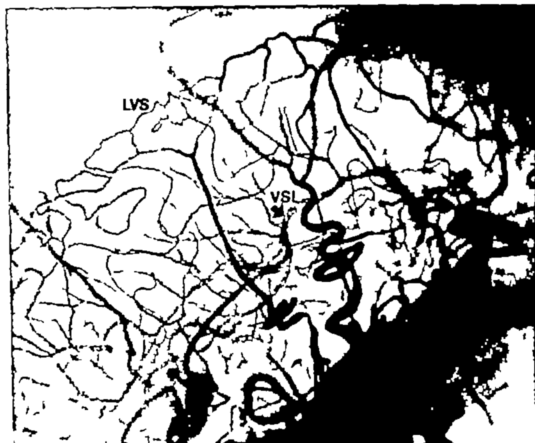


Fig 34 Guinea pig, spiral limbus, basal-second turn, transverse section. Radiating arterioles with vascula spring-coils supplying the spiral limbus. An irregular peripheral margin, the limbus vessels (LVS), is formed by the arcades. Collecting venules drain the limbus vessels, often terminating in the vein of the spiral lamina (VSL). Note the capillary net centrally in the spiral limbus (95X)

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Fig 35 Guinea pig, spiral limbus, basal turn, transverse section, phase contrast. Peripherally the limbus vessels form an irregular margin. Note open sparse loops of the capillaries centrally Arrow = Huschke dents, i.e. limbus cells (186X).

Peripherally in the tympanic lip and below the habenula perforata, parallelly and centrally to VSBM lies the other marginal vessel the *vessel of the tympanic lip* (VSTL, Vas labii tympanici) (Fig 31 32, 33) The supplying and draining radiating vessels meet VSTL within 15 degrees of the right angle. In the first quarter of the basal turn VSTL is replaced by capillary arcades which lack a spiral marginal appearance. In the second quarter these arcades and VSTL can be demonstrated simultaneously (Fig 32) From the second half of the basal turn VSTL can be demonstrated to the apex. Apically it differs from the vessel of the basilar membrane in having more frequent interruptions and in running more unevenly Particularly in the apical turn, short radiating connections from VSTL regularly communicate with the vessel of the basilar membrane. The diameter of the two marginal vessels is about 5 microns with VSTL usually being the larger of the two The diameter is well maintained in the three basal turns for both marginal vessels (Fig 37 Table IV) The region between the two mar

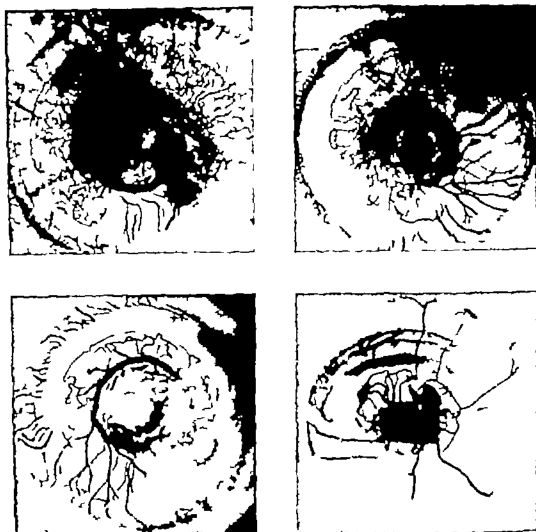


Fig. 36 Guinea pig, spiral lamina, a) basal turn (above left), b) 2nd turn (above right), c) 3rd turn (below left) d) apical turn (below right). The rich vascular supply of the basal turn is already reduced in the second turn. Principal arrangement maintained. Discontinuities in the marginal vessel increase in frequency. In the third turn the arrangement is still maintained with radiating and marginal vessels. The vascular arrangement is more open and sparse. Apically in the fourth turn the vascular arrangement is very simplified with sparse radiating vessels turning off at the level of the marginal vessels and forming simple arcades. (a 27X, b 29X, c 33X, d 39X).

ginal vessels constitutes a relatively avascular zone which follows the spiral course of the cochlea and which is more or less of constant breadth in the three basal turns (Fig. 36 37 Table IV) The few vessels which are found in this area are those supplying the vessel of the basilar membrane Since the absolute area of the spiral lamina decreases apically the avascular region between the two marginal vessels occupies a relatively larger portion here

The radiating arterioles *supplying the limbus vessels* (LVS, *Vasa limbi*) have the same course and derive from the same vessels in the modiolus as those supplying the spiral lamina (Fig 31 34) Different branches from the same arterioles and venules are provided for the marginal vessels and the LVS, but

there are no direct connections between the marginal vessels and the LVS. The LVS are situated immediately peripherally to the attachment of the vestibular membrane and make the spiral limbus one of the most vascularized regions in the cochlea. No cell in this region is further than 30 microns from a nearby vessel. The LVS are formed of short spirally-running parts of the arcades with an irregular looping course (Fig. 34, 35). The diameter of the LVS is about 4—5 microns and somewhat smaller than that of the marginal vessels (Fig. 37, Table IV). The angles of the radiating vessels when these ramify to the LVS are a little less than the right angle. Immediately peripherally to the LVS lie the so-called Huschke's dents indicating the presence of the limbus cells (Fig. 35). More centrally in the spiral limbus there is an arrangement of vascular loops (Fig. 35). It is important to note that the LVS belong to the capillary regions in the wall of the scala media.

The *collecting venules* (CVL, Venulae collectae) draining the two marginal vessels and the limbus vessels run centrally to the modiolus. They then continue basally on the modiolus wall to meet the spiral modiolar vein on its apical aspect or to the vestibulo-cochlear vein (Fig. 13, 14). Centrally in the spiral lamina the finest ramifications of the radiating arterioles and the CVL contribute to a capillary net, but larger anastomoses also occur between the arterioles and the CVL. In the basal turn the CVL empty into one (or two) *vein of the spiral lamina* (VSL, V. laminae spiralis), which has a spiral course (Fig. 31, 32). VSL receives branches from the spiral lamina and limbus, spiral ganglion, acoustic nerve and the wall of the modiolus.

The *vestibular* and *tectorial membranes* are completely avascular regions in adult guinea pigs. Contrarily to the human cochlea, no vessels have been demonstrated in the basilar membrane between the vessel of the basilar membrane and the external wall of adult guinea pigs.

The vascular arrangement described in the spiral lamina and limbus is principally valid for the whole length of the cochlea. There is, however, a marked simplification of the vascular architecture in the apical parts (Fig. 36) where the marginal vessels and the limbus vessels form very simple arcades. In the apical turn the marginal vessels are often replaced by single arcades in which it is no longer possible to differentiate between the two vessels.

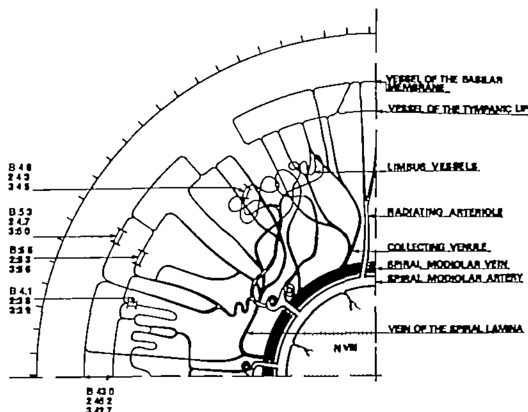


Fig 37 Guinea pig, spiral lamina, transverse section, schematic, measurements |=| = diameter of vessels, ←— —→ = distance between vessels.

TABLE IV

Measurements in the spiral lamina and limbus

	Basal	T rn 2nd	3rd	SD[1]	Significant Differences
a. Distance between VSBM and VSTL	43.0	45.2	42.7	9.6	A T = T = T
b. Fractions f essels supplying VSBM[2] VSBM / (VSBM + VSTL)	24.4 /	23.5	26.3		A T = T = T
c. Vessel diameters					
VSBM	5.3	4.7	5.0	1.8	A T = T = T
VSTL	5.5	5.3	5.6	2.0	A T = T = T
capillaries[3]	4.1	3.8	3.9	1.7	A T = T = T
limbus essels	4.8	4.3	4.5	1.7	A T = T = T

All me surement in micron
[1]) SD Standard deviation (error).
[2]) Two me surements per turn and animal.
[3]) The f nert mifications of the adiating rterioles and collecting enules were measured mmedi t ly en rall to VSTL.
VSBM h essel f the b la membran
VSTL = the essel of the tympanic l p.
A = Statistically significant d fference bet ween nimal
T = statistically significant difference bet ween turns in he same animal.
(T = basal turn, T = second turn, T = th rd turn)



Fig 38 Man, spiral lamina, basal turn, transverse section. Note the incomplete injection. Arterioles and venules radiate over the spiral lamina supplying the marginal vessels, i.e. the vessel of the basilar membrane (VSBM) and the vessel of the tympanic lip (VSTL). VSBM is continuous and curves evenly in spiral direction. VSTL is discontinuous. Arrow = isolated branch radiating to the venules at the basilar membrane, CVL = collecting venules of the scala tympani (47X).

b *Man*

The preparation of the spiral lamina in the human cochlea is considerably more difficult than in the guinea pig While in the latter it is easily detached from the external wall, in man the attachment is much stronger and the spiral lamina must be cut off from the external wall between the vessel of the basilar membrane and the venules at the basilar membrane, preferably without destroying these structures.

Principally the vascular arrangement is similar in man and the guinea pig, and the same vascular structures can be demonstrated (Fig 31 38)

Radiating arterioles (RAL, Arteriolae radiatae) and *collecting venules* (CVL, Venulae collectae) radiate at right angles from the artery and veins

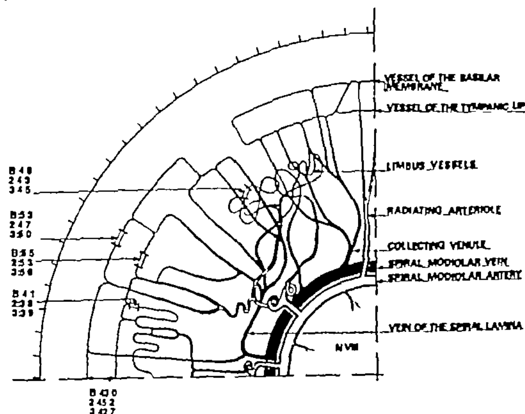


Fig 37 Guinea pig, spiral lamina, t ansverse section schematic, measurements |—| = diameter of vessels, ←— —→ = distance between essels.

TABLE IV

Measurements in the spiral lamina and limbus

	Basal	Turn 2nd	3rd	SD[1]	Significant Differences
a. Distance between VSBM and VSTL	43.0	45.2	4..7	9 6	A T = T = T
b. Fractions of vessels supplying VSBM[2]	4 4 /	23.5	26.3		A T = T = T
VSBM / VSBM + VSTL					
Vessel diameters					
VSBM	5.3	4.7	5.0	1.8	A T = T = T
VSTL	5.5	5.3	5.6	2.0	A T = T = T
capillaries[3]	4 1	3.8	3 9	1.7	A T = T = T
limbus essels	4 8	4.3	4.5	1.7	A T = T = T

All measurements in microns.

) SD = Sta dard deviation (error)

[2]) Tw measurements per turn and animal

[3]) The finest ramifications f the radiating arterioles and collecting enules were measured mmediately centrally to VSTL.

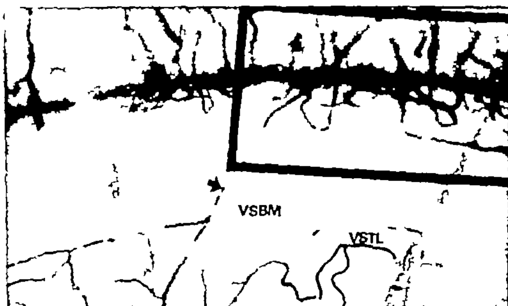
VSBM = he essel of the basilar membrane

VSTL = the vessel of the tympanic lip.

A = Statistically ignificant difference between a imals.

T statistically significa t difference between rns in he same animal.

(T = basal turn, T = second turn, T = h d turn).



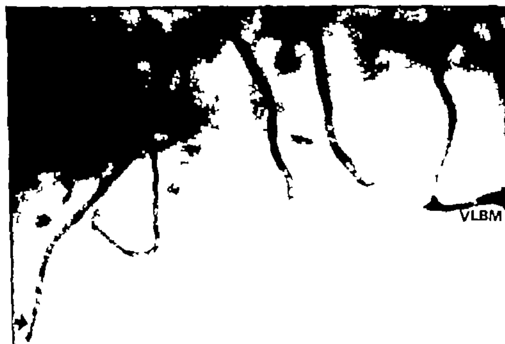


Fig. 40. Man, spiral lamina, second turn, transverse section. The framed area above represents the region demonstrated below. VSBM = the vessel of the basilar membrane, VSTL = the vessel of the tympanic lip, arrow = radiating vessel crossing over the otherwise vascular area peripheral to VSBM and connecting to the vessel at the basilar membrane (VLBM) (above — 98X, below — 186X)

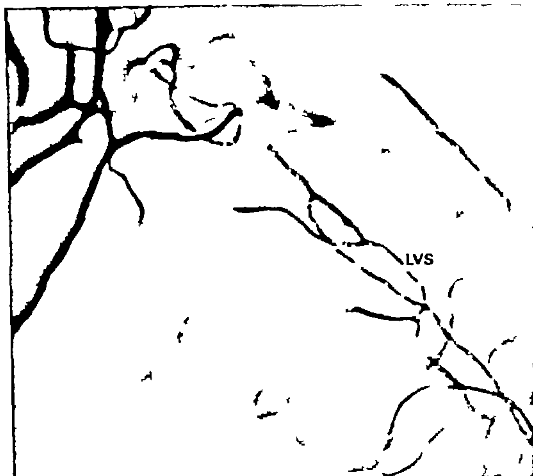


Fig 41 Man, spiral limbus, basal turn, transverse section. Radiating arterioles and collecting enules supplying the limbus essels (LVS) which form a spiral arcadic margin peripherally in the spiral limbus (155X)

In some cochleas *connections between vessels of the spiral lamina and vessels in the external wall* have been demonstrated In these cases isolated branches from the radiating or marginal vessels continue through the otherwise avascular region between VSBM and the external wall where they connect with the venules at the basilar membrane (Fig 38 40)

The spiral limbus is supplied by continuous arcadic vessels the *limbus vessels* (LVS Vasa limbi) (Fig 41) The supplying arterioles and draining venules turn at more or less right angles to meet the LVS The LVS follow a somewhat straighter course and comprise a looser network than the irregular looping formations observed in the guinea pig

The same trends toward simplification of vascular structures are found in *apical parts of the cochlea* in both the guinea pig and man. Thus in the spiral lamina the vessel of the basilar membrane is more or less continuous to the apex while many interruptions are found in the vessel of the tympanic lip (Fig. 42, 43) Arterioles and venules supplying the marginal and limbus vessels decrease in number apically and the arcades of the limbus vessels become successively more open.

Fig. 42. Man, spiral lamina, third turn, tranverse section. Incomplete injection. The vascular arrangemen is maintained with radiating arterioles (RAL) nd collecting venules (CVL) the vessel of the basilar membrane (VSBM), and discontinuous essel of the tympanic lip (VSTL) (95X).

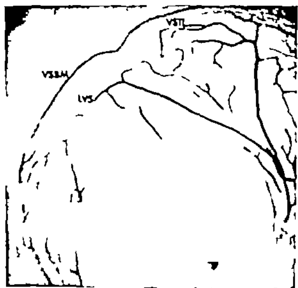


Fig. 43 Man, spiral lamina helicotrema, pical turn, transverse section. The principal vascular arrangement is maintained with sparse open arcades and spirally-running vessel of he basilar membrane (VSBM) the essel f the tympanic lip (VSTL) and the limbus essels (LVS) (53X)

c. *Comment*

Three names have been added to the sparse vascular anatomical nomenclature in this region the *vessel of the basilar membrane* the *vessel of the tympanic lip* and the *limbus vessels* In general the vessel of the basilar membrane formerly has been called vas spirale or the spiral vessel (Table VIII) This designation may give the erroneous impression, previously widely held, that the blood flow in this single vessel is spiral from the base toward the apex However the vascular arrangement very clearly demonstrates that the radiating vessels to the two marginal vessels are alternately arterioles and venules. The blood flow thus may run in opposite directions for short spiral distances (Fig 84) Another important consideration is that the two named vessels constitute a spiral vascular margin peripherally to which a principally avascular zone is situated. Thus the vessel of the basilar membrane and the vessel of the tympanic lip have been referred to as *marginal vessels* The suggested specific names of the marginal vessels and the limbus vessels are consistent with the proposed nomenclature in other parts of the cochlea i e that the vessels be named according to their anatomical position and relationship to other vascular structures.

The vascular arrangement in the spiral lamina is composed of three separate arcadic systems of which the limbus vessels are situated centrally to and above the marginal vessels. The limbus vessels belong to the wall of the scala media and make the spiral limbus a highly vascularized region, particularly in the guinea pig The two marginal vessels might be considered as belonging rather to the boundary of the scala tympani Rich anastomosing possibilities exist between radiating arterioles and collecting venules.

The measurements have demonstrated that the distance between the two marginal vessels is well maintained apically in spite of the decreasing volume of the scalas (Table IV) Although the vascular arrangement becomes less dense apically the diameter of the capillary vessels is nevertheless well maintained. Measurements of the angles between radiating vessels and the marginal vessels demonstrate that the deviation from the right angle is greater for connections to the vessel of the basilar membrane than to the vessel of the tympanic lip. Centrally in the spiral lamina, the ramifications of the radiating vessels show rather obtuse angles, especially when dividing to the limbus vessels.

The only measurements available for comparison with those from the present investigation are to be found in Table II page 20 The values found in the present study for the diameter of the vessel of the tympanic lip and the limbus vessels (Table IV) compare well with those of Iurato Charachon, however found a greater diameter for the vessel of the basilar membrane in man than those found in the present investigation for the guinea pig

The similarity between the vascular anatomy of man and the guinea pig concerning the spiral lamina and spiral limbus is striking The arrangement of the limbus vessels is very similar In both the guinea pig and man there is a pronounced simplification of the vascular architecture toward the apex. Vessels of all types become successively fewer show more frequent interruptions and

fewer anastomoses. Differences, however do appear In man the vessel of the basilar membrane is continuous whereas in the guinea pig interruptions are frequent. The network of the limbus vessels comprises a simpler and more sparse arcadic system in man than in the guinea pig The vessel of the tympanic lip is more defined in the guinea pig than in man.

Another interesting observation concerning the vascular anatomy of the spiral lamina is the lack of vessels peripherally to the vessel of the basilar membrane. However connections between the vessels of the spiral lamina and the external wall were demonstrated in a few human preparations in the present investigation Only Smith (1954) mentions these connections and then only briefly These structures might be a remnant from embryonic life (Bredberg, 1968) and are probably to sparse to be of any functional significance. With this exception found only in man, the periphery of the spiral lamina as well as the vestibular and tectorial membranes are completely avascular The vessel of the basilar membrane is probably of greater importance during embryonic life when it is considerably larger than in the adult (Boettcher 1887 Bredberg, 1968)

It is interesting to note that the vascular arrangement in the spiral lamina is principally similar to that of the external wall if the latter is imagined folded so that the arterioles and venules radiate from the same common center as they do in the spiral lamina. In both structures there are spiral vascular systems at right angles to the radiating vessels. In the external wall there are several spiral systems in all three scalas without many anastomoses between them. In the spiral lamina, however there are frequent and regular anastomoses between the marginal vessels. There are no direct connections between these and the limbus vessels.

The vascular anatomy of the external wall has been well studied both *in vivo* and *in vitro* However the anatomy of the spiral lamina seems to be rather unknown. From the technical point of view *in vivo* studies of the vascular anatomy of the spiral lamina are extremely difficult to perform. It is probable that further investigations of the vessels in the spiral lamina will confirm that they are of the same importance as those of the external wall.

3 *The vascular anatomy of the external wall of the membranous cochlea*

a *Guinea pig*

There are five different types of vessels in the *scala vestibuli* (Fig 44)
Radiating arterioles
Collecting venules
The vessel of the scala vestibuli
The vessel at the vestibular membrane
A capillary net above the vestibular membrane

The *radiating arterioles* (RAL, Arteriolae radiatae) are branches from the spiral modiolar artery which peripherally to the "vascular spring-coils" in the modiolus, pass to the most apical parts of the scala vestibuli They then continue radially along the external wall of the membranous part of the scala vestibuli (Fig 45) The distance between two individual RAL varies greatly (Fig 46) and in general increases toward the apex (Table V) The diameter of the two branches resulting from a ramification may differ considerably Such divisions most commonly occur in the modiolus above the attachment of the vestibular membrane and in the wall of the scala media (Fig. 47) The RAL in the

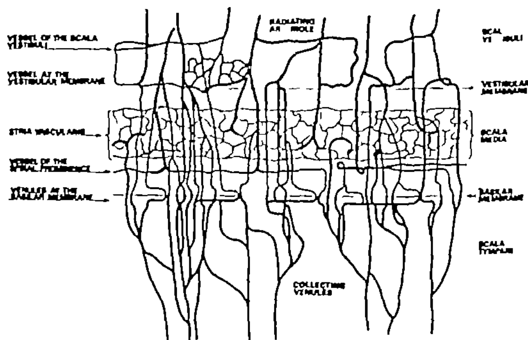


Fig 44 Guinea pig and man, external wall, radial section, schematic. Radiating arterioles predominate in the scala vestibuli and supply spiral vascular systems the vessel of the scala vestibuli, the vessel at the vestibular membrane, the stria vascularis, and the vessel of the spiral prominence Collecting venules predominate in the scala tympani and drain spiral capillary vessels and the capillary net in the scala vestibuli A spiral vessel is formed basally to the basilar membrane by the venules at the basilar membrane. Radiating arterioles and collecting venules connect with one another by arterio-venous anastomoses lying externally to the stria vascularis.



Fig 43 Guinea pig cochlea with surrounding bone. Semi-transparency allows identification of the vessels in the external wall. OW = oval window, RW = round window VRW = vein of the round window Note the simplification of the vascular arrangement at the apex (30X).

external wall are observed to ramify 0—7 times, the mean number being 3—4 In general the number of ramifications successively decrease apically The angle at the ramification of the RAL is usually somewhat smaller (cia 70 degrees) than that between the RAL and the spiral vessels (usually close to 90 degrees) The branches join all types of vessels in the external wall (Fig 44 47) In the basal turn most of the branches from the RAL connect with the collecting venules as arterio-venous anastomoses which run externally to the stria vascu-

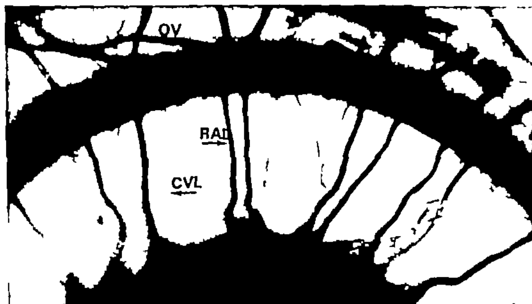


Fig 46 Guinea pig, scala estibuli, basal-second turn, tra sverse section, RAL = radiati g arterioles of the scala estibuli in the basal turn, CVL = collecting enules of the scala tympani in the second turn, arrows = delicate collecti g enules of the sc la estibuli in th basal turn, OV = osseous essels (95X).

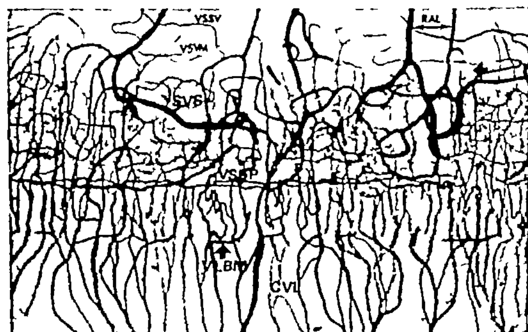


Fig 47 Guinea pig, ex ernal wall, basal turn, radial section, RAL = radiating rterioles, VSSV = essel f the scal estibuli, VSVM = vessel t the estibula membrane, SVS = stria vascularis, VSSP = essel f he spiral prominence, VLBM = venules t the basilar membrane CVL = collecting enules Arrow indicat the ttachment of the vestibula membrane and the attachment of the basilar membrane (76X)

TABLE V

Distances and vascular diameters in the external wall of the guinea pig cochlea

	Base	Turn 2nd	Turn 3rd	SD[1]	Significant Differences
a. breadth of the stria vascularis	251.8	164.8	113.3	24.7	AI T >T >T
b. basal marginal vessel of stria vascularis — vessel of the spiral prominence	59.8	59.1	58.7	15.9	T =T =T
c. distance between two radiating arterioles at the level of the vessel of the scala vestibuli	109.8	125.5	187.4	90.3	T =T <T
d. distance between two collecting venules in the scala tympani[2]	20.2	31.8	44.2	18.7	T <T <T
e. diameter of the vessel of the scala vestibuli	4.6	4.7	5.0	1.3	AI T =T =T
f. diameter of the vessel at the vestibular membrane	5.1	5.9	4.9	2.9	A T =T =T
g. diameter of the capillaries in the stria vascularis	4.5	4.4	5.5	2.2	T =T <T
h. diameter of the vessel of the spiral prominence	7.1	6.0	6.7	2.4	A T =T =T
i. diameter of the radiating arterioles at the level of the vessel of the scala vestibuli	12.1	9.3	10.1	3.0	AI T >T =T
j. diameter of the arterio-venous anastomoses externally to the stria vascularis	6.6	6.3	6.4	2.1	AI T =T =T
k. diameter of the collecting venules[1]	9.8	8.7	8.7	3.2	T =T =T
l. vascular density in cm/cm³ of the stria vascularis[3]	297.1	293.6	300.0	40.9	T =T =T

All measurements in microns
[1]) SD = standard deviation (error)
[2]) measured immediately below the venules at the basilar membrane
[3]) two measurements per turn in each animal
A = statistically significant difference between animals
I = statistically significant interactions
T = statistically significant difference between turns in the same animal
(T = basal turn, T = second turn, T = third turn)

laris. The stria vascularis and the vessel of the spiral prominence are supplied by the proportionally largest branches. The mean diameter of the RAL in the three basal turns is respectively 12, 9 and 10 microns (Fig. 48 Table V)

The number of RAL was measured in 10 cochleas in the stereo-microscope as apically as possible in each scala vestibuli, i.e. close to the joint connecting the scala vestibuli to the next scala tympani. The number of radiating arterioles diminishes significantly with each turn (Fig. 49 Table VI)

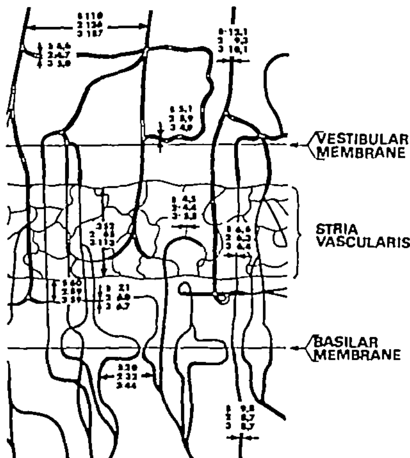


Fig 48 Guinea pig, external wall, radial section, schematic. Measurements in microns. ←— —→ = distance between vessels, —→ ←— = diameter of vessel

Isolated, delicate *collecting venules* (CVL, Venulae collectae) are found in the scala vestibuli at least in the basal turn (Fig 44 46) Branches from these venules and the radiating arterioles take part in the formation of the vessel of the scala vestibuli and the vessel at the vestibular membrane as well as a sparse *capillary net* most prominent between these two vessels (Fig 50) The CVL pass radially parallel to the radiating arterioles but in an apical direction. They then empty into the spiral modiolar vein in the turn immediately apical Occasionally

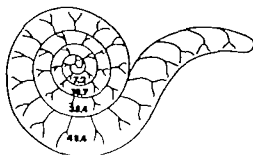


Fig 49 Guinea pig cochlea viewed from above, schematic. Number of radiating arterioles/turn in the scala vestibuli.

TABLE VI

The number of radiating arterioles/turn in the scala vestibuli of the guinea pig cochlea

Turn	Mean	Standard Deviation
Base	48.4	3.90
Second	35.4	1.62
Third	18.7	1.27
Fourth	7.2	1.25

The results are the mean of measurements on 10 cochleas examined under the stereo-microscope at the joint between the scala vestibuli and the next scala tympani. Statistically significant differences between all turns.

the CVL take a spiral course for some distance just before entering the spiral modiolar vein (Fig. 46)

A vessel which has hitherto not been described is the one here called the *vessel of the scala vestibuli* (VSSV Vas scalae vestibuli) VSSV describes a spiral course, in the basal turn 130 microns, in the second and third 80 microns above and parallel to the attachment of the vestibular membrane (Fig 44 47 50) VSSV is not continuous but nevertheless is well-defined for intervals, especially in the basal turn. In the apical turns VSSV has a more serpentine course and occurs less frequently VSSV generally connects directly with the vessel at the vestibular membrane and the venules in the scala tympani without previously ramifying. The diameter of VSSV is relatively constant in the three basal turns (Fig. 48 Table V)

Another spiral vessel lies parallel to and immediately above the attachment of the vestibular membrane: the *vessel at the vestibular membrane* (VSVM, Vas membranae vestibularis) Similarly to VSSV it is formed by branches of the radiating arterioles and collecting venules turning off spirally at about right angles (Fig. 44 47 50) VSVM can be demonstrated in the whole cochlea, but is best developed in the basal turn VSVM has no anastomoses with the stria vascularis or the vessel of the spiral prominence, but does connect with collecting venules in the scala tympani. The distance from VSVM to the vestibular membrane increases somewhat in the apical turns. The diameter of VSVM is around 5 microns.

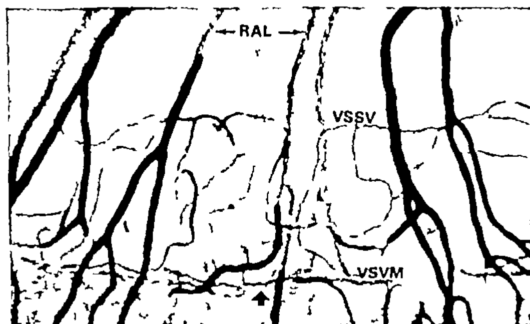


Fig. 50 Guinea pig, scala vestibuli, basal turn, radial section. RAL = radiating arterioles, CVL = collecting venules, VSSV = vessel of the scala vestibuli, VSVM = vessel of the vestibular membrane, arrow = attachment of the vestibular membrane. A capillary net is formed between VSVM and VSSV (above — 149X, below — 155X).

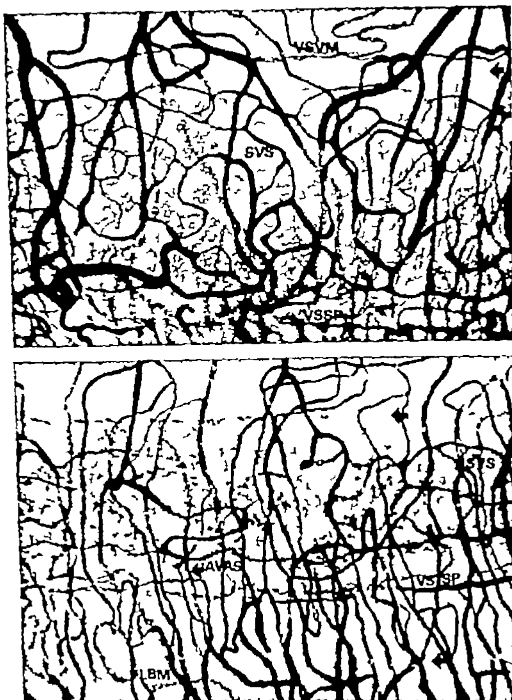


Fig. 31 Guinea pig, scala media, radial section. Above: basal turn. A vascular net, stria vascularis (SVS), is formed by capillaries of different sizes. Arrow = attachment of the vestibular membrane, VSVM = vessel at the vestibular membrane, VSSP = vessel of the spiral prominence. Below: second turn. Stria vascularis is incompletely injected. Note the arterio-venous anastomoses externally to the stria vascularis. Arrows = attachment of the vestibular membrane and the attachment of the basilar membrane, AVAS = arterio-venous anastomoses, VSSP = vessel of the spiral prominence, VLBM = vessels at the basilar membrane (above — 149X, below — 155X)

In the external wall of the *scala media* there are three different vascular structures (Fig 44)

Stria vascularis

The vessel of the spiral prominence

Arterio-venous anastomoses

A great deal of the external wall of the scala media is covered by the *stria vascularis* (SVS) which consists of a rich net of vessels forming closed polygonal loops (Fig. 44 47 51) At least in the basal turn there are well defined spirally running apical and basal marginal vessels. SVS narrows markedly apically and in the fourth turn only sparse open loops are left (Fig. 48 52, Table V) The vascular arrangement in the apex is so sparse as to scarcely deserve the name vascular net. However the distance between nearby vascular loops is similar in the whole cochlea lying within the range 40—60 microns.

In spite of the decreasing breadth of the SVS the ratio capillary length/tissue area remains the same in the three basal turns (Table V) The mean diameter of these capillaries is similar in the basal and second turns (4.5 microns) but is greater in the third turn (5.5 microns) (Fig 48 Table V) Relatively few of the radiating arterioles in the scala vestibuli supply the SVS and relatively few collecting venules in the scala tympani drain it, but those which do are of comparatively large diameter (Fig 47) These connecting arterioles and venules derive from a region external to the SVS and usually terminate in the middle of the vascular net. The SVS has no other vascular anastomoses than these. Unfortunately it is commonly experienced that, even in preparations which are well injected in all other regions, some of the capillaries in the SVS have not been filled.

Parallel to and below the stria vascularis runs another spiral vessel in the spiral prominence the *vessel of the spiral prominence* (VSSP Vas prominentiae spiralis) (Fig 44 47 53) Similarly to the stria vascularis it is supplied by few but relatively large branches from the radiating arterioles and collecting venules connecting at rather long intervals in a T formed manner Short sections of VSSP often appear to be formed by two and even three vessels. VSSP is well developed in the whole cochlea including the apical turns (Fig 52) Its diameter is similar in the three basal turns and is somewhat greater than that of the capillaries of the stria vascularis (Fig 48 Table V) VSSP has no vascular connections with the stria vascularis and the distance between them remains constant in the three basal turns (Fig 48 Table V)

In the basal turn most of the radiating arterioles supply neither the stria vascularis nor the VSSP, but instead ramify and merge externally to these structures with the collecting venules of the scala tympani to form *arterio-venous anastomoses* (AVAS, Anastomosis arteriovenosa) (Fig 49 51) There are frequent ramifications of the AVAS at rather small angles (cia 60 degrees) and the resulting capillary branches are of greater diameter than those of the stria vascularis but similar in size to the vessel of the spiral prominence. Some unramified larger branches are found in the basal turn In the apical turns the

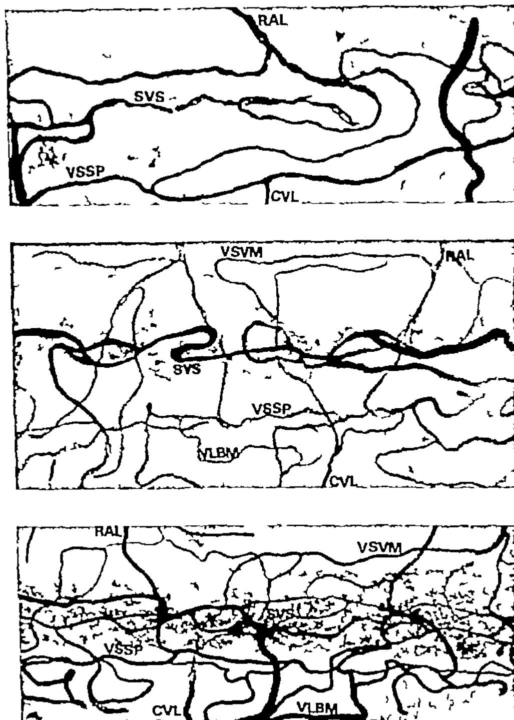
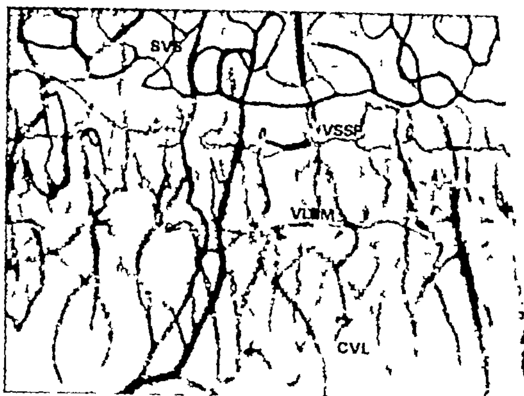


Fig. 52. Guinea pig, external wall, radial section. Below: basal portion, third turn. Middle: apical portion, third turn. Above: fourth turn. The principal arrangement is maintained. Vasculature is simplified, stria vascularis (SVS) narrows. There are few arterio-venous anastomoses. At the apical end (above) the vascular components are greatly simplified and difficult to identify. RAL = radiating arterioles, CVL = collecting venules, VSVM = vessel at the vestibular membrane, VSSP = vessel of the spiral prominence, VLBM = venules at the basilar membrane (below — 144X, middle — 150X, above — 186X).



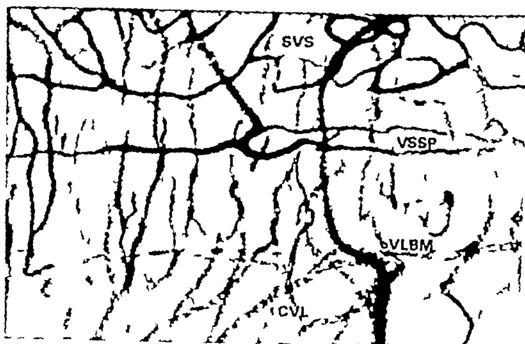


Fig. 53. Guinea pig, external wall, basal turn, radial section. SVS = stria vascularis, VSSP = vessel of the spiral prominence (at times double or discontinuous), VLBM = venules at the basilar membrane, CVL = collecting venules, arrow = attachment of the basilar membrane (below — 213X, above — 133X)



Fig 54 Guinea pig, scala tympani, basal turn, radial section. Collecting venules (195X).

number of these AVAS decreases more than other types of vessels in the external wall (Fig. 52)

In the external wall of the *scala tympani* there are two types of vessels (Fig 44)

Collecting venules
The venules at the basilar membrane

The *collecting venules* (CVL) constitute the drainage for all vessels in the external wall (Fig 44 47 51 53 54) and anastomose with one another The CVL have a particular arrangement at the attachment of the basilar membrane immediately above it they turn off short spirally make an omega formed loop around the attachment and continue in their former apico-basal direction In this manner the venules seem to "grip" around the attachment of the basilar membrane. Below this attachment the *venules at the basilar membrane* (VLBM, Venulae membranae basilaris) form a spiral vessel (Fig. 44 47 53) VLBM can be demonstrated in all four turns and are only a spiral part of the CVL. The diameter of the CVL is similar in the three basal turns and the distance between them increases apically Small venules collect to form larger ones basally in the scala tympani. The ramification angles of the CVL in the scala tympani are about



Fig 55 Guinea pig, dome of the cochlea viewed from above Spiral modiolar artery terminates apically by ramifying to form radiating arterioles (RAL) supplying the dome and extending over the external wall (78X).

50 degrees. In the apical turns the number of CVL and the number of their ramifications decreases. Due to the lack of an appropriate reference level in the scala tympani, the number of CVL was not counted as was done for the radiating arterioles in the scala vestibuli. The general impression is that there are at least as many CVL as radiating arterioles.

In the apical turns of the cochlea the vascular arrangement is principally maintained as it is described here There is, however a pronounced simplification of the whole vascular anatomy (Fig 52) The vessel of the scala vestibuli can be demonstrated for intervals in the third turn but disappears in the fourth The stria vascularis consists of sparse open loops. The arterio-venous anastomoses frequently observed in the basal turn also disappear in the fourth turn where all radiating arterioles join some of the spiral vascular systems. In the fourth turn the radiating arterioles thus turn off at right angles in a spiral direction at the level of some of these spiral vessels, run for some distance spirally and again turn basally to the collecting venules. The dome of the cochlea is supplied by the finger like terminal ramifications of the spiral modiolar artery (Fig 55) The measurements confirm many of these observations (Table V VI VII) The number of arterio-venous anastomoses and radiating arterioles and their ramifications decreases Instead the vessel at the vestibular membrane and the vessel of the spiral prominence are supplied by twice as many radiating arterioles in the fourth as in the basal turn The number of radiating arterioles supplying the stria vascularis increases less. In general there is no change in the occurrence of vascular connections in the external wall apically

b *Measurements*

The distribution of the radiating arterioles to different vessels in the external wall

The vascular anatomy and the measurements presented give a general idea of the vasculature in the external wall. There are, as demonstrated, several spiral vessels or spiral vascular systems at right angles to the radiating arterioles and collecting venules. These are

The vessel of the scala vestibuli
The vessel at the vestibular membrane
Stria vascularis
The vessel of the spiral prominence
The venules at the basilar membrane

Some of the arterial branches, however run to the venous side in the scala tympani without supplying any of these spiral systems. These are the arterio-venous anastomoses which also were considered in the measurements. Those branches lying closest to the stria vascularis usually turn to the collecting venules above the attachment of the basilar membrane Vessels lying more externally pass by the venous "pitchforks" at the attachment and connect to the venules more basally in the scala tympani The terminations of the arterio-venous anastomoses thus were considered separately under "venules above" and "venules below" the attachment of the basilar membrane. As it is difficult to separate the vessel of the scala vestibuli from the vessel at the vestibular membrane in the fourth turn, the supplying vessels to these two were measured together The results of the measurements are presented in Fig. 56 and Table VII and are expressed in terms

TABLE VII

The percentage distribution of the radiating arterioles to different vessels in the external wall of the guinea pig cochlea

	Base	2nd	3rd	4th
Vessel of the scala vestibuli	7 /	8	13 /	40 / (combined with next row)
Vessel at the vestibular membrane	13	13	20 /	
Stria vascularis	13 %	10 /	16	19 /
Vessel of the spiral prominence	11 /	16	19	24 /
Venules above the attachment of the basilar membrane	34	29	23	15 %
Venules below the attachment of the basilar membrane	22 /	24 /	9 /	2 %
	100 /	100	100	100 %

The results are the mean of measurements taken in 10 cochleas under the stereo-microscope. All the ramifications of 10 radiating arterioles were followed in each turn and the figures give the percentage of the total number supplying each vascular entity

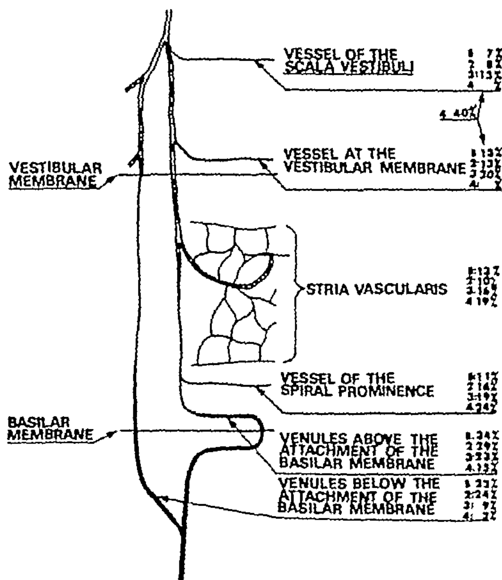


Fig. 56. Guinea pig, external wall, radial section, schematic. Measurements. Percentage distribution of the arterioles to different vessels. In the basal turn the arterio-venous anastomoses predominate, decreasing apically. Anastomoses occurring above and below the attachment of the basilar membrane are grouped separately.

of percentages. The measurements did not include the diameter of the two further branches resulting from a ramification, which may vary considerably.

The results demonstrate a rather even distribution of the radiating arterioles to all vessels. It is important to note that the stria vascularis and the vessel of the spiral prominence are supplied only by comparatively few branches. The greatest change apically is, as described, the marked decrease of the arterio-venous anastomoses. This decrease corresponds to a rather even increase of connections with the spiral vessels apically. The importance of the vessel of the scala vestibuli and the vessel at the vestibular membrane is emphasized by the fact that these each receive about the same number of supplying vessels as the

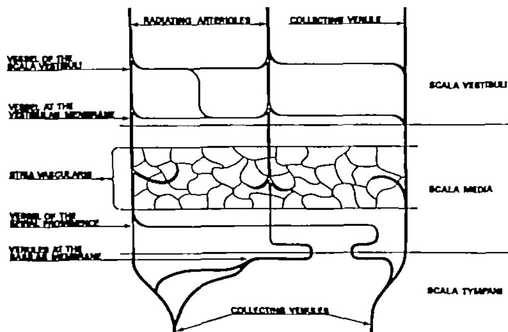


Fig. 57 Guinea pig and man, external wall, radial section, schematic. Vascular connections. Radiating arterioles and collecting venules connect with all types of vessels. Stria vascularis and the vessel of the spiral prominence only connect to supplying arterioles and draining venoles. The venules at the basilar membrane form spirally-running vessel.

stria vascularis and the vessel of the spiral prominence in the two apical turns, even though the latter two vessels are supplied by larger branches. The number of branches supplying the stria vascularis is rather constant although it increases somewhat apically. In this connection, it is well to again note that the vascular density of the stria vascularis is maintained at least to the third turn in spite of the visible thinning out of the vascular net (Table V). Both the vessel at the vestibular membrane and the vessel of the spiral prominence receive as many or more supplying vessels as the stria vascularis, demonstrating the importance of a good vascular supply in these regions.

Vascular anastomoses in the external wall

The existence of vascular anastomoses between the vessels in the external wall was confirmed visually and photographically. The results are presented schematically in Fig. 57. The radiating arterioles supply all kinds of vessels in the external wall, and the collecting venules in the scala tympani drain all vessels. Stria vascularis and the vessel of the spiral prominence have only connections with these arterioles and venules and no connections with one another. In this respect they differ from the other spiral systems. The vessel of the scala vestibuli and the vessel at the vestibular membrane thus connect with all types of vessels except the stria vascularis and the vessel of the spiral prominence. The venules at the basilar membrane constitute a spirally running part of the collecting venules.

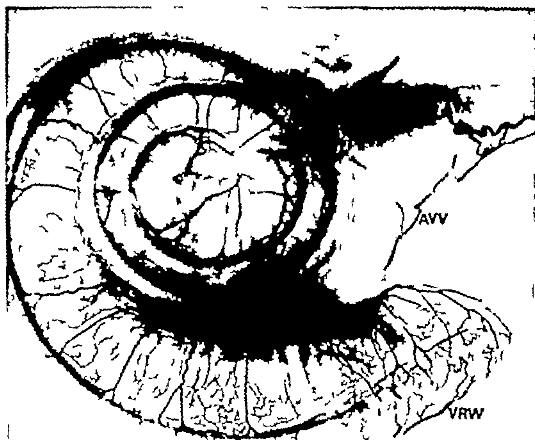


Fig 58 Human cochlea. Radiating arterioles in the scala vestibuli are darker injected than the veins. A sparse capillary net is formed in the scala vestibuli. VRW = vein of the round window AVA = anterior vestibular artery AVV = anterior vestibular vein (12X).

c. *Man*

In the external wall of the *scala vestibuli* four types of vessels can be demonstrated (Fig 44)

Radiating arterioles
Collecting venules
The vessel at the vestibular membrane
A capillary net above the vestibular membrane

Generally the *radiating arterioles* (RAL, *Arteriolae radiatae*) appear darker than the collecting venules and at least centrally have a larger diameter (Fig 58) Similarly to the RAL supplying the spiral lamina, the RAL leave the artery in the modiolu at about right angles. The RAL centrally have a pronounced winding course but further peripherally in the scala vestibuli they straighten (Fig 59) Centrally in the scala vestibuli branches forming spirally running arcades connect the RAL with one another Larger arterioles sometimes deviate

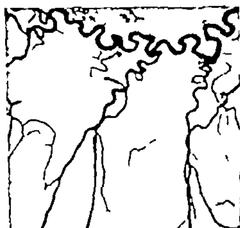


Fig 59 Man, modiolus and external wall, basal turn, transverse section. Radiating ar terioles are given off from the artery which runs around the modiolus. They first ha winding course but straighten further peripherally Partially-injected arcades forming connections between the radiating arterioles can be seen (27X)

Fig 60 Man, external wall, basal turn, radial section. Radiating arterioles (RAL) supply all kinds of essels in the external wall. A capillary net of the scala estibul is seen, basally bordered by the vessel t the vestibular membrane (VSVM). CVL = collecting venules of the scala vestibul and the scala tympani, SVS = stria vascularis, typically only partially injected, VSSP = vessel f the spiral prominence, AVAS = rterio-venous anastomoses (103X)

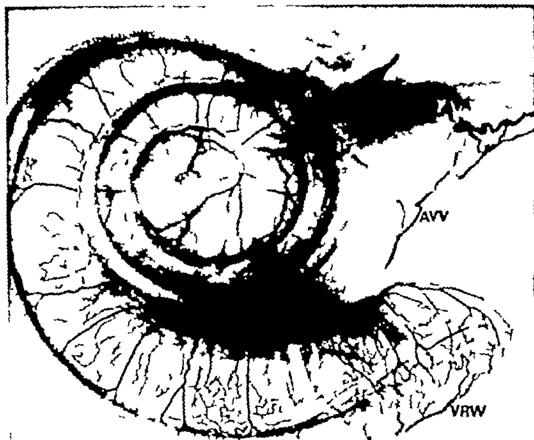


Fig 58 Human cochlea. Radiating arterioles in the scala vestibuli are darker injected than the veins. A sparse capillary net is formed in the scala vestibuli. VRW = vein of the round window AVA = anterior vestibular artery AVV = anterior vestibular vein (12X).

c *Man*

In the external wall of the *scala vestibuli* four types of vessels can be demonstrated (Fig 44)

Radiating arterioles
Collecting venules
The vessel at the vestibular membrane
A capillary net above the vestibular membrane

Generally the *radiating arterioles* (RAL, Arteriolae radiatae) appear darker than the collecting venules and, at least centrally have a larger diameter (Fig. 58) Similarly to the RAL supplying the spiral lamina, the RAL leave the artery in the modiolu at about right angles The RAL centrally have a pronounced winding course but further peripherally in the scala vestibuli they straighten (Fig 59) Centrally in the scala vestibuli branches forming spirally running arcades connect the RAL with one another Larger arterioles sometimes deviate

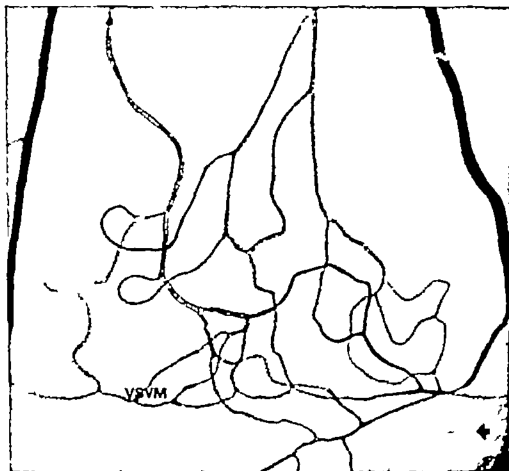


Fig. 62. Man, external wall, basal turn, radial section, scala vestibuli. Two darkly injected radiating arterioles and two lightly injected collecting venules of smaller diameter. A capillary net, formed by the branches of both, is basally lined by a spiral vessel: the vessel at the vestibular membrane (VSVM). Arrow = attachment of the vestibular membrane (91X).

buli are considerably more frequent in man than in the guinea pig. As in the guinea pig the number of radiating arterioles and CVL decreases markedly in the apical turns (Fig. 63). Similarly to the radiating arterioles, larger branches of the CVL are sometimes seen to deviate spirally in the scala vestibuli (Fig. 25).

The *capillary net* in the scala vestibuli forms a basal marginal vessel lying close and parallel to the attachment of the vestibular membrane: the *vessel at the vestibular membrane* (VSVM, Vas membranae vestibularis) (Fig. 60, 62, 63). There is a similar and corresponding vessel in the guinea pig. On the other hand there is no vessel in man corresponding to the vessel of the scala vestibuli in the guinea pig. VSVM connects the arterioles and venules in the scala vestibuli and further the venules in the scala tympani, the latter often by direct unramified anastomoses. VSVM is well formed in the whole cochlea, including the apical turn (Fig. 63).

Fig. 63. M... rn l wall ... di l section. Below: middle ... second ... rn. Middle: basal portion ... h d ... rn. Above: ... pi l portion ... hird turn. The principal arrangement is maintained, but mo... pl f d ... d open. The ... essel ... f the spiral prominence (VSSP) follows ... straighter course. Th... sc l ... (SVS) ... arrows. RAL ... rad... ting arterioles, VSVM = vessel ... he vestibul... VLBM ... en les ... he basil... membrane, CVL ... collecting ven les. (95X)

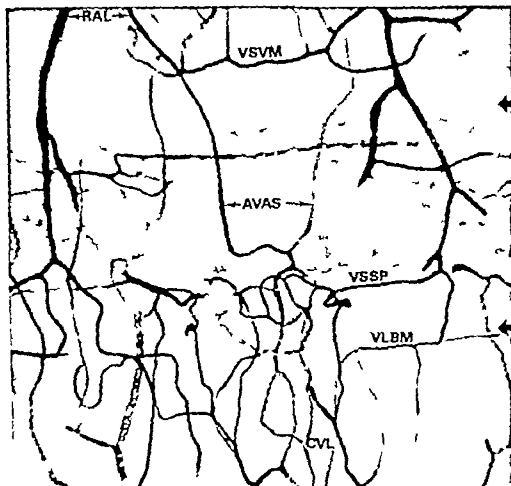


Fig. 64. Man, external wall, basal turn, radial section. Stria vascularis (corresponding here to the pigmented area) is typically difficult to inject. It appears that the contrast is being shunted past the stria vascularis to the other vessels. RAL = radiating arterioles, VSVM = vessel at the vestibular membrane, AVAS = arterio-venous anastomoses, VSSP = vessel of the spiral prominence, VLBM = venules at the basilar membrane, CVL = collecting venules. Arrows indicate attachment of the vestibular and basilar membranes (95X).

Three different vascular structures can be demonstrated in the external wall of the *scala media* (Fig. 44)

Stria vascularis
The vessel of the spiral prominence
Arterio-venous anastomoses

The *stria vascularis* (SVS) proved to be particularly difficult to inject in the human cochlea. While in a few preparations it is completely injected, in many others the contrast was apparently shunted directly over to the venous system (Fig. 64). In some cases the SVS seems to have been filled in a retrograde direction from the venules in the scala tympani (Fig. 61). As in the guinea pig, the only vascular connections are radiating arterioles from the scala vestibuli, and

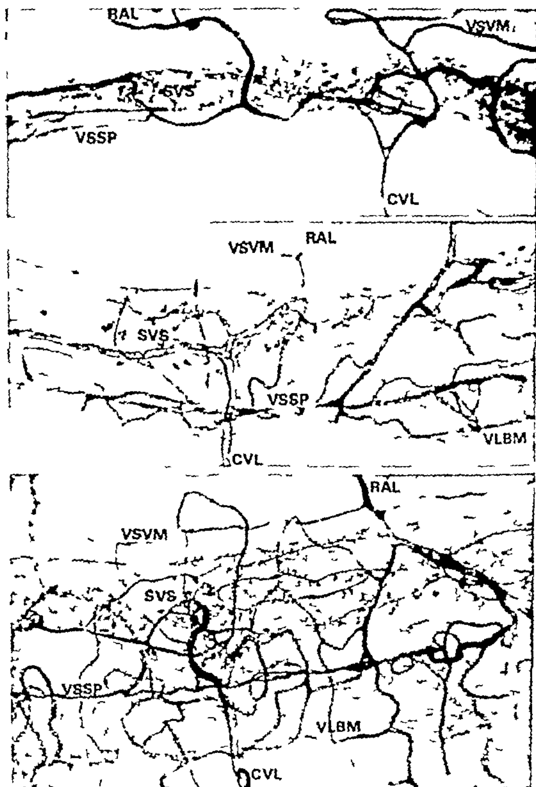


Fig 63. M... external wall, radial section. Below: middle of second turn. Middle: basal portion ... third turn. Above: ... portion, third turn. The principal arrangement is maintained, but ... more simpl...ed and open. The vessel of the spiral prominence (VSSP) follows a straighter course ... (SVS) narrows. RAL = radiating arterioles, VSVM = vessel ... the vestibular memb..., VLBM = venules ... the basilar membrane, CVL = collecting venules (95X)

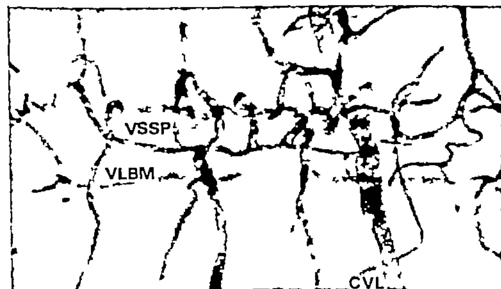


Fig 66 Man, external wall, radial section. The vessel f the spiral prominence (VSSP), single or double, has an uneven course and is often complicated by supplying or draining vessel ramifying or looping immediately before arriving. SVS stria vascularis, VLBM vessels at the basilar membrane, CVL = collecting venules (155X)



Fig 67 Man, external wall, basal turn, radial section. Collecting venules of the scala tympani with frequent anatomoses (95X).

Two different vascular structures can be identified in the external wall of the *scala tympani* (Fig 44)

Collecting venules
The venules at the basilar membrane

The *collecting venules* (CVL, Venulae collectae) pass around the attachment of the basilar membrane form a spiral vessel below the attachment here referred to as the *venules at the basilar membrane* (VLBM, Venulae membranae basilaris) and turn again basally in the scala tympani (Fig 63 64 66) VLBM can be demonstrated in the whole cochlea and, as in the guinea pig, they constitute a spiral part of the CVL. The CVL drain all the spiral vascular systems in the external wall Basally to the VLBM the venules collect to form larger vessels (Fig 67) which eventually empty into the vein of the scala tympani or the common modiolar vein (see chapter Modiolus)

In the *apical turn* the vascular arrangement is principally maintained as in the guinea pig There is, however a marked reduction in the number of vessels and ramifications and a general simplification. The dome is supplied by the terminal ramifications of the spiral modiolar artery (Fig 63 68)

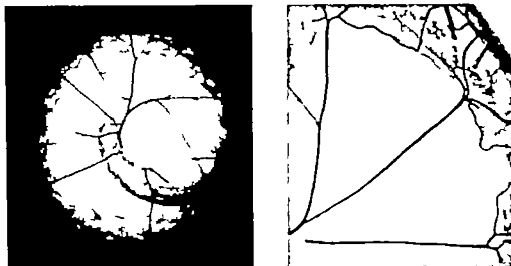


Fig. 68. Man, second and third turns. Left: viewed from inside toward the helicotrema. Right: viewed from outside on the dome. Radiating arterioles ramify little compared with the base (17X, 29X)

d. *Comment*

The commonly accepted nomenclature has been applied to the radiating arterioles, collecting venules, arterio-venous anastomoses, stria vascularis and the vessel of the spiral prominence. The *vessel of the scala vestibuli* found in the guinea pig but not in man, hitherto has not been described and is here classified among the other spiral vessels. In addition, the seldomly described *vessel at the vestibular membrane* and the *venules at the basilar membrane* were demonstrated in both man and the guinea pig in the present study and were accordingly given names.

Principally the vascular arrangement in the external wall of the cochlea radiates apico-basally over the radiating arterioles, arterio-venous anastomoses and collecting venules. Several spiral vascular systems are found at right angles to these vessels, i.e. the vessel of the scala vestibuli, the vessel at the vestibular membrane, stria vascularis, the vessel of the spiral prominence and the venules at the basilar membrane. In the basal turn the radiating vessels predominate and give the impression that the main blood flow passes this way. The relatively small angles at the ramifications of the radiating arterioles and collecting venules as well as observations *in vivo* confirm this impression. The angles between the radiating and spiral vessels are throughout greater.

From the literature one receives the impression that all the radiating arterioles join the stria vascularis and the vessel of the spiral prominence. However, the present investigation indicates that only a minor part of the radiating arterioles connect to these systems, even though they often may be branches of comparatively large size. As demonstrated, there are no vascular connections between the stria vascularis and the vessel of the spiral prominence. In addition, each is

provided with a separate system of supply and drainage vessels. The measurements indicate that the distance between them is rather small and unchanged in the three basal turns. An interesting fact is that the hitherto neglected vessels, the vessel of the scala vestibuli and the vessel at the vestibular membrane receive a considerable part of the radiating arterioles.

Reservations have been raised concerning the measurements of the diameter of vessels. Unfortunately very few previous measurements are available for comparison. Values obtained by previous workers are tabulated in Table II page 20 It is often difficult to make meaningful comparisons between figures presented in different works due to the variation in nomenclature and methodology In general it can be said that the measurements of the present investigation compare well with those given by other authors on all types of vessels presented by them except the capillaries of the stria vascularis which were larger in the investigations of Perlman et al (Table II) Iurato s figures are relatively small and the values given by Charachon for man are uniformly larger than those found in the present investigation.

The measurements confirm the apparent apical simplification of the vascular anatomy in the guinea pig Table V shows a considerable and statistically significant increase in the distance between pairs of radiating arterioles and collecting venules apically as compared with the base The number of radiating arterioles in the scala vestibuli decreases markedly and they ramify less. The figures from the present investigation (Table VI page 67) may be compared with those of Nabeya

	Basal	Second	Third	Fourth
Nabeya:	34	20	16	7
	30	20	14	6
Present nvestigation	48	33	19	7

Nabeya s values are smaller than those of the present investigation, but are difficult to compare as it was not stated exactly where the measurements were made, but it seems to be in the same region

The measurements further demonstrate a statistically significant decrease in the breadth of the stria vascularis apically

However these measurements should be related to the decreasing volume of the turns apically If the number of radiating arterioles in the scala vestibuli in the present investigation is related to the length of each turn as given by Fernandez (1952) the number of arterioles per mm is respectively 5.7 7 4 5.5 3 4 In spite of the visibly increasing vascular sparseness of the stria vascularis, the total length of the capillaries per surface unit is similar in the three basal turns. The increasing sparseness is further well compensated for by the well maintained diameter of the vessels apically The measurements also demonstrate that in the apical turns almost all of the branches of the radiating arterioles connect to some of the spiral vascular systems and the arterio-venous anastomoses decrease markedly

It thus appears that the seemingly increasing sparseness of the vascular anatomy apically is not so great if the occurrence and length of the vessels is related to the length and the volume of the turn. The greatest difference between the vasculature basally and apically would appear to be that the simplified vascular system apically allows for fewer possibilities of adjustment in the event of circulation variations.

The similarity of the vascular anatomy in the guinea pig and man is striking. Only the vessel of the scala vestibuli present in the guinea pig is not found in man. Furthermore, the venules in the scala vestibuli are more numerous in man where they take part in the formation of a well developed capillary net. With these exceptions it can be stated that the occurence, appearance and course of the vessels is largely similar in the guinea pig and man. These principal similarities are further evident when compared with the vascular anatomy as described in the rat, dog, cat and monkey (Asai, 1908; Nabeya, 1923; Smith, 1954).

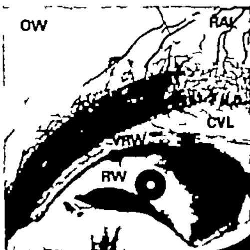


Fig 69 Guinea pig, basal end. The radiating arterioles (RAL) in the scala vestibuli run more obliquely as do the collecting e- nules (CVL) i the scala tympani when emptying into the ei of the round win dow (VRW). The vascular arrangement narrows toward the estibulum. OW — oval window RW = round window (34X).

Fig 70 Guinea pig, basal end, radial section. All vascular structures can be identified and the principal arrangement is maintained. Radiating arterioles (RAL) run more obliquely VSVM = vessel at the estibular membrane, SVS = stria vascularis, VSSP = essel f the spiral prominence, VLBM = enules t the basilar membrane, VRW = vein f the round window receiving collecting enules running obliquely in the scala tympani, OW = oval window RW = round window (95X)

4 *The vascular anatomy of the basal end of the cochlea*

a. *Guinea pig*

By the basal end of the cochlea is meant the partially uncoiled terminal segment of the cochlea which turns from a plane perpendicular to the modiolar axis to a plane almost parallel to this axis. The same vessels can identified as are found in the rest of the cochlea and in general the vascular arrangement is maintained.

In the *external wall* the radiating arterioles in the *scala vestibuli* deriving from the spiral modiolar artery do not have a straight apico-basal course but rather a more oblique one toward the basal end over the scala vestibuli (Fig 69 70) They do not supply the extreme basal end of the external wall which is supplied instead by 3—4 arterioles coming from the opposite direction, i.e the vestibulum (see further below) In general both the vessel of the scala vestibuli and the vessel at the vestibular membrane are easily demonstrated, although the former is not found in the above-mentioned extreme basal region (Fig. 70, 75) In the *scala media* the stria vascularis gradually narrows toward the basal end, but nevertheless remains a well maintained vascular network (Fig 70 75) The vessel of the spiral prominence is well formed and the arterio-venous anastomoses externally to the stria vascularis are numerous (Fig 70 75) The collecting venules of the *scala tympani* resemble the radiating arterioles in the scala vestibuli in having an oblique course (Fig 70) They empty into the

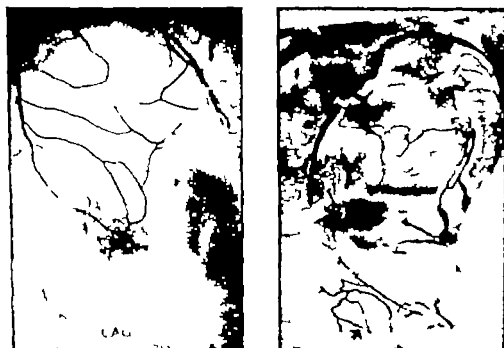


Fig 71 Guinea pig, basal end, round window Note the capillary net on the round window membrane The round window is 3/4 encircled by the [illegible] of the round window Arrow = periosteum [illegible], CAQ = cochlear aqueduct (left — 39X, right — 52X)

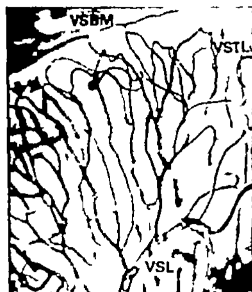


Fig 72 Guinea pig, basal end, spiral lamina, transverse section. The principal vascular arrangement is maintained, but the vessel of the tympanic lip (VSTL) is replaced by arcades (arrow) in the most basal part. VSBM = vessel of the basilar membrane, VSL = vein of the spiral lamina (81X).

Fig 73 Guinea pig, extreme basal end. Serpentine radiating arterioles (RAL) and collecting venules (CVL) coming from the modiolus turn around the extreme basal end of the spiral lamina and turn to the basal termination of the vessels in the external wall. Stria vascularis (SVS) and the vessel of the spiral prominence (VSSP) connect with venules. The arterioles supply the scala vestibuli. VSBM = vessel of the basilar membrane (95X).

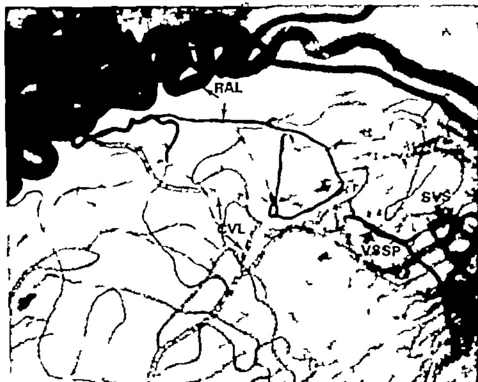


Fig. 74. Guinea pig, extreme basal end. A sparse capillary net is formed at the extreme basal end. RAL = radiating arterioles, CVL = collecting venules, SVS = stria vascularis, VSSP = vessel of the spiral prominence (152X).

vein of the round window (VRW V. fenestrae cochleae) which turns around the apical aspect of the window while gradually approaching the scala media (Fig. 70, 71, 75, 77). The collecting venules in the scala tympani are correspondingly shorter in this region. VRW encloses about 3/4 of the round window and eventually empties into the vestibulo-cochlear vein (Fig. 77). The round window membrane is supplied by a sparse net of anastomosing branches of the VRW (Fig. 71). The venules at the basilar membrane are generally easily demonstrated (Fig. 70, 75) and the venules "gripping" the attachment of the basilar membrane are particularly numerous.

In the *spiral lamina* the vessel of the basilar membrane is generally completely continuous, but the vessel of the tympanic lip is not demonstrable and is replaced by numerous arcades located centrally to its normal position (Fig. 72). The limbus vessels follow the general pattern found in the remaining parts of the cochlea.

The *extreme basal end* of the external wall is supplied by arterioles deriving from the vestibulo-cochlear artery in the vestibulum. They pass the basal end of the spiral lamina and turn apically to the scala vestibuli (Fig. 73, 74). A short anastomosing vascular net is formed where these arterioles meet the terminal ramifications of the spiral modiolar artery coming from the opposite direction. This interesting anastomosing region, which has not been described before, is particularly prominent in the scala vestibuli (Fig. 75).

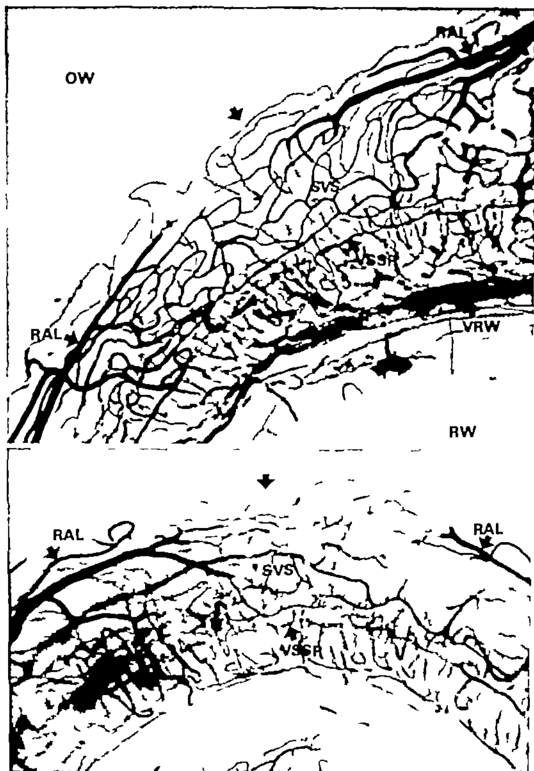


Fig 75 Guinea pig, basal end, radial section. Photos from two different animals showing the interesting capillary net (arrow) formed in the scala vestibuli where the radiating arterioles (RAL) from the vestibulum (left) meet the radiating arterioles coming from the opposite direction (right). The principal arrangement is otherwise maintained. OW = oval window, RW = round window, SVS = stria vascularis, VSSP = vessel of the spiral prominence, VRW = vein of the round window (above — 93X below — 95X)

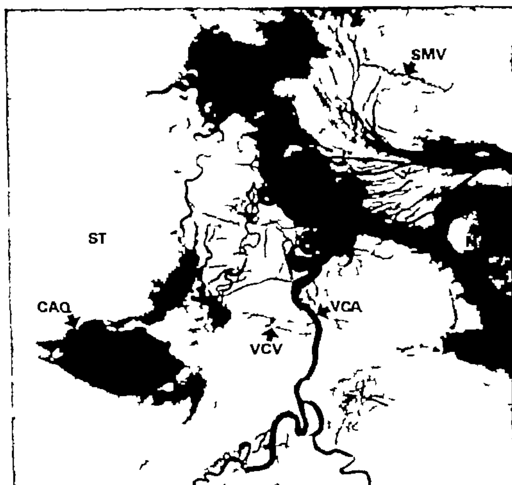


Fig. 76. Guinea pig, base-vestibulum, radial section. The scala tympani (ST) at the basal end showing the origin of the cochlear aqueduct (CAQ). SMV = spiral modiolar vein, VCA = vestibulo-cochlear artery VCV = vestibulo-cochlear vein, N VIII = acoustic nerv (49X).

Small venules deriving from the vestibulo-cochlear vein also accompany the larger radiating arterioles around the basal end of the spiral lamina (Fig. 73 74) They drain terminating regions of the stria vascularis, the vessel of the spiral prominence, and the collecting venules in the scala tympani at the extreme basal end. Branches from these arterioles and venules form a sparse irregular vascular net at the extreme basal end of the spiral lamina (Fig 74)

The *vestibulo-cochlear vein* (VCV V vestibulo-cochlearis) crosses the vestibulum perpendicular to the corresponding artery (Fig 76) VCV receives the vein of the round window and drains the extreme basal end of the spiral lamina and the external wall, in particular the vestibulum and superior parts of the labyrinth

The *vein of the cochlear aqueduct* (VCAQ, V aqueductus cochleae) is formed by the confluence of the spiral modiolar vein and the vestibulo-cochlear vein (Fig. 17 77) VCAQ begins below the round window runs a short course in the

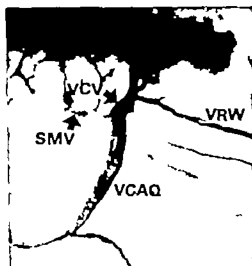


Fig 77 Guinea pig. Vein of the cochlear aqueduct (VCAQ) in the temporal bone is formed by the confluence of the vestibulo-cochlear vein (VCV) and the spiral modiolar vein (SMV) VCV is shown receiving the vei of the round window (VRW). (22X)

temporal bone close and parallel to the aqueduct, and empties into the jugular vein It receives some venous branches from the temporal bone in this region some of which can be followed to a niche below the scala tympani near the round window where the osseous vessels connect with mucoperiosteum vessels. VCAQ provides the main outflow for the whole membranous cochlea and probably also for some of the vessels of the surrounding mucoperiosteum and bony capsula

b. *Man*

The vascular anatomy of the basal end of the cochlea in man is principally similar to that of the guinea pig (Fig 22, 29 78) The main stem of the vestibulo-cochlear artery divides into two branches upon its arrival in the modiolus, and the vestibular branch supplies the spiral lamina and the external wall in the basal end It then runs toward the vestibulum with an uneven serpentine course and upon arriving is situated between the sacculus and the basal end of the spiral lamina (Fig 79 80) Branches are given off in both directions to the capillary regions and some branches turn around the basal end of the spiral lamina and reverse their direction apically to supply the most basal parts of the external wall (see further below)

The basal end of the human cochlea is drained by the vestibulo-cochlear vein or more properly by its two branches: the vein of the round window and the posterior vestibular vein (Fig 29) The *vestibulo-cochlear vein* (VCV V vestibulo-cochlearis) begins close to the basal end as the confluence of the anterior and posterior vestibular veins (Fig. 29 78) The *anterior vestibular vein* (AVV V vestibuli anterior) drains parts of the vestibulum and the labyrinth. The *posterior vestibular vein* (PVV V vestibuli posterior) runs together with the vestibular branch of the vestibulo-cochlear artery from the vestibulum,

Fig. 7[illegible] [illegible]



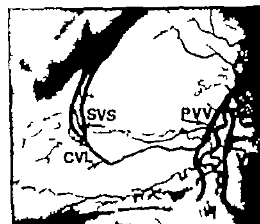


Fig 79 Man, basal end, radial section, viewed from estibulum. Vestibular branch (VB) of the vestibulo-cochlear artery running toward the vestibulum together with the posterior vestibular vein (PVV). When arriving at the extreme basal end of the spiral lamina, radiating arterioles (RAL) reverse direction and supply vessels in the external wall. Collecting venules (CVL) drain the extreme basal end of the stria vascularis (SVS). ST = scala tympani, SV = scala vestibuli, N VIII = acoustic nerv (abov — 13X, below — 22X).

draining the spiral lamina and the scala vestibuli (Fig 78 79 80) Venous and arterial branches accompany one another around the basal end of the spiral lamina (see further below) The vestibulo-cochlear vein also receives a small segment of the vein of the scala vestibuli running in the opposite direction to the posterior vestibular vein This vein supplies the spiral lamina and the external wall in the region corresponding to the cochlear branch of the vestibulo-cochlear artery but it can not always be demonstrated (Fig 29 78)

The *vein of the round window* (VRW V fenestrae cochleae) resembles the corresponding vein in the guinea pig (Fig 29 78 81 82) It runs on the apical aspect of the round window enclosing 3/4 of it, and anastomoses in the region of the extreme basal end with the venous branches from the posterior vestibular vein running around the basal end of the cochlea. VRW receives short venules from the scala tympani at the basal end and eventually empties into the vesti-

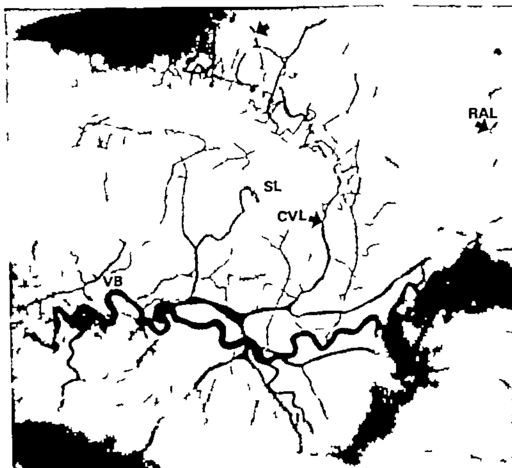


Fig 80 Man, basal end, transverse section. As in the guinea pig, radiating arterioles (RAL) and collecting venules (CVL) turn around the extreme basal end of the spiral lamina (SL) and turn to the extreme basal end of the external wall. Arrow = venules turning to the scala vestibuli, VB = vestibular branch f the vestibulo-cochlear artery (29X).

bulo-cochlear vein. Usually no vessels could be demonstrated on the human round window membrane, in contrast to the guinea pig

The *vein of the cochlear aqueduct* (VCAQ, V aqueductus cochleae) is formed by the confluence of the vestibulo-cochlear vein and the common modiolar vein. This region lies near the round window below the scala tympani (Fig 28 29 78) VCAQ runs through the temporal bone closely parallel to the cochlear aqueduct. In most preparations it receives several osseous veins during its course to the basal aspect of the temporal bone where it empties into the jugular vein.

In the *external wall* the arterioles, instead of radiating perpendicularly over the *scala vestibuli* take a more oblique course toward the basal end similarly to the collecting venules in the *scala tympani* (Fig. 81 82) The radiating arterioles and collecting venules in the scala vestibuli form a capillary net basally bordered by the vessel at the vestibular membrane.

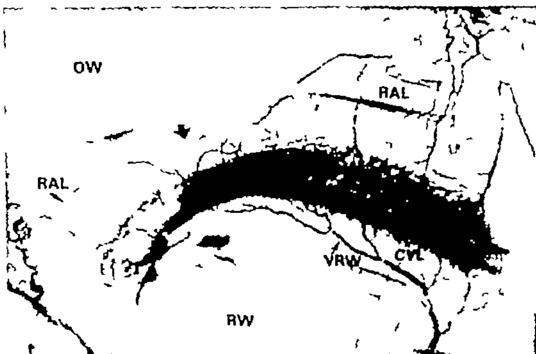


Fig 81 Man, basal end, radial section. Radiating arterioles (RAL) taking more oblique course. Collecting venules (CVL) of the scala tympani taking a short oblique course before emptying into the vein of the round window (VRW). Radiating arterioles (RAL) coming around the extreme basal end of the spiral lamina and turning retrograde to the scala vestibuli, forming an anastomosing net with arterioles coming from the opposite direction (arrow). OW = oval window RW = round window (25X)

In the *scala media* of the basal end as in the whole cochlea, stria vascularis is difficult to inject. There exists here a distinct difference between the guinea pig and man in that the human stria vascularis narrows to a very sparse net and often to only one or two vessels at the basal end (Fig 79 82) The most pronounced simplification, however is not found at the extreme basal end but rather somewhat more apically Stria vascularis in the extreme basal end usually consists of at least two parallel vessels with interconnecting branches (Fig. 83). The extreme basal end of the external wall is supplied by ramifications from the vestibular branch of the vestibulo-cochlear artery and from the posterior vestibular vein passing the basal end of the spiral lamina in a "retrograde" direction (Fig 78 79 80 81) At the extreme basal end of the spiral lamina the posterior vestibular vein receives venules from both the scala vestibuli and scala tympani as well as some ramifications constituting the extreme basal termination of the stria vascularis and the vessel of the spiral prominence (Fig 79) As in the guinea pig, an anastomosing region thus occurs in the scala vestibuli where the arterioles from the vestibulum meet those coming from the opposite direction In this particular region the stria vascularis is especially poorly developed (Fig 81 82) The vessel of the spiral prominence is quite irregular at the basal end and this region is often difficult to inject. The vascular arrangement of the *spiral lamina* is similar to the rest of the cochlea (Fig 80)

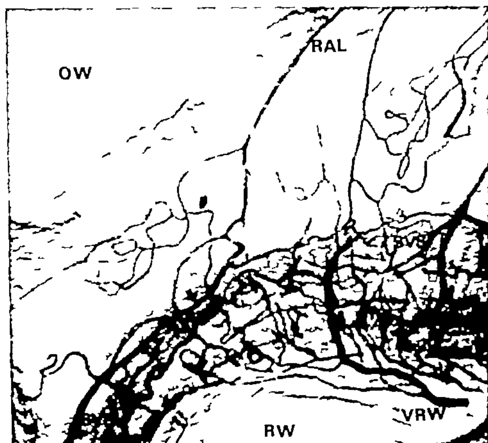


Fig 82 Man, basal end, radial section. Radiating arterioles (RAL) with an oblique course over the scala vestibuli anastomosing with radiating arterioles coming from the vestibulum (left). A *small capillary net is formed where they meet*. In this region stria vascularis (SVS) is particularly poorly developed (arrow). OW = oval window. RW = round window. VRW = vein of the round window (95 X).

c. *Comment*

The basal end is distinguished from other areas of the cochlea by a markedly different anatomy probably due to its unique position between the oval and round windows and to the fetal development. The vascular anatomy of this region has not been described previously in detail. It should be emphasized that this area in man is supplied by branches from the same artery which, however come from opposite directions. Thus, when the basally running artery arrives at the basal end of the spiral lamina branches turn around this pivot point and run in a retrograde direction apically. An anastomosing net is formed where the branches of the same artery meet. The accompanying venous ramifications similarly make the turn at the basal end, eventually draining the extreme basal

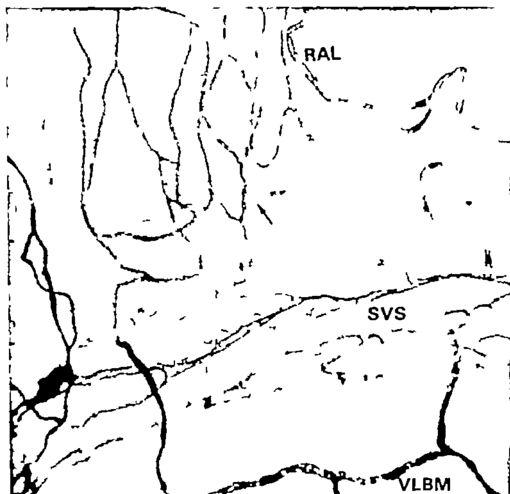


Fig 83 Man, extreme basal end, radial section A capillary net (arrow) is formed at the extreme basal end in the scala vestibuli. Stria vascularis (SVS) is greatly simplified. The venules at the basilar membrane (VLBM) connect with a radiating arteriole (RAL) and the capillary net in the scala vestibuli (95X)

end of the stria vascularis and the vessel of the spiral prominence. They also connect with venules in the scala vestibuli and the scala tympani

This interesting arrangement at the basal end is thus largely similar in man and the guinea pig with a few exceptions. The vestibulo-cochlear artery supplies a comparatively larger part of the basal end in man than in the guinea pig The vessels on the round window membrane found in the guinea pig are seldom demonstrated in man and the stria vascularis in this region is rather different in the two species as mentioned above The guinea pig stria vascularis is well formed in the extreme basal end in spite of progressive narrowing In man, even though the general vascular arrangement in this region is well maintained the stria vascularis appears poorly developed, especially at the level where the radiating arterioles in the scala vestibuli anastomose. The vascular anatomy of the spiral lamina is principally similar to the rest of the cochlea.

C. GENERAL DISCUSSION

Nomenclature

In the present investigation the most commonly used nomenclature has generally been employed. It is felt that the attitude toward the introduction of new names must be one of great restrictivity However it has been considered necessary to suggest new names in certain cases. Firstly this was done with hitherto not described or named vessels. Secondly some names were changed to more descriptive ones indicating the localization of the vessel in relation to anatomical structures as well as to suggest a more uniform nomenclature. Some of these previous terms have been misleading as well as insufficiently descriptive

The new names introduced in the present investigation are given in the table below (for a more complete survey see Table VIII page 124)

Present investigation	*Previously called*
Spiral modiolar artery (Guinea pig and man)	Cochlear artery A. cochleae propria
Common modiolar vein (man)	No name available
Vein of the scala vestibuli (man)	Anterior spiral vein
Vein of the scala tympani (man)	Posterior spiral vein
Vessel of the basilar membrane	Spiral vessel Vas spirale
Vessel of the tympanic lip	Table VIII
Limbus vessels	" "
Vessel of the scala vestibuli (guinea pig)	No name available
Vessel at the vestibular membrane	Table VIII
Venules at the basilar membrane	" "

The method

The guinea pig was chosen as the experimental animal since a large amount of morphological and physiological research has been carried out on this animal, allowing one to put the findings of the present investigation in relationship to previous studies.

One of the aims of the present investigation was to refine and simplify the method of contrast injection. The present method proved to have the following advantages

a. The injection with Berlin blue solution admits the demonstration of all vascular areas in the cochlea in the guinea pig as well as in man

b. The method is relatively fast, requiring one week including all steps of preparation.

c. Glycerin was used as the storage medium with the advantage of preserving the elasticity of the preparations, in being transparent and nonvolatile and in not visibly changing the contrast in the vessels.

d By focusing at different levels in the stereo-microscope, all vessels could easily be followed in the decalcified transparent tissues.

e. The method permits general surveys, detailed analysis of the vascular anatomy as well as measurements of vascular areas.

The present method is basically similar to previously employed methods of contrast injection All these methods have their disadvantages, e.g in not giving uniformly injected vessels In addition, previous authors do not discuss the relative importance of the various steps in their procedures. The present method often failed to yield reproducible injection of all cochlear vessels in spite of extensive experimentation with variations in an attempt to overcome these defects. As in previously published methods, the present one includes many steps in the preparation, each of which must be carefully controlled to minimize variation in the final product. A rigorous documentation and statistical analysis of all variations in technique is thus neither practical nor particularly illuminating The variations in methodology which were introduced were empirical modifications based on experience acquired during the elaboration of the method. An important weak point seems to be the vessels of the inner acoustic meatus. A relatively high injection pressure is required to pass these vessels and to fill all the capillaries of the cochlea with contrast, yet they are easily ruptured by extreme pressure The safety margin between these two limiting factors seems to be small

The varying results of the perfusion with contrast can be turned, however to the researcher's advantage in certain cases. In this way independent observation of the arterial and venous systems is permitted in those instances when only one of them is filled. In addition, the study of certain areas is facilitated when covering parts are not injected, and a better survey of the larger vessels may be obtained when the capillaries are not filled with contrast.

Other techniques for the demonstration of the cochlear vessels have also given valuable information, in particular vascular studies *in vivo* Such observations have the advantage of being more physiologically relevant and have given interesting findings in vascular experiments. For the study of the vascular anatomy the *in vivo* technique is less appropriate as only small parts of the external wall are exposed. The injection of contrast medium probably gives the most complete overall survey of the vascular anatomy as well as opportunities for detailed studies.

Comments on previous investigations

The findings of previous authors will not be discussed in detail here. The intention is not to compile a list of differences between the findings of previous authors and the present study but rather to present a few examples of the nature of these differences

The studies by Eichler (1892) and Siebenmann (1894) may be considered the starting point for modern investigations of the cochlear blood vessels. Their descriptions and drawings mainly treat the larger vessels and provide a general

picture of the vascular anatomy The descriptions of the venous system given by these two authors are probably essentially correct, but are difficult to interpret. Greater divergencies from the present investigation are found concerning the arterial system. Eichler does not describe the vestibulo-cochlear artery and Siebenmann's description of the tractus spiralis arteriosus is unclear Eichler maintains that the capillary net in the scala vestibuli is merely arterial, and Siebenmann considers the scala tympani to have an arterial supply Both of these views are probably incorrect. Capillary regions are in general only briefly treated.

The findings of the present investigation are in general agreement with the works of Nabeya (1923) Smith (1951 1954) Scuderi & del Bo (1952) and Charachon (1961) Significant divergencies do occur however and certain vascular areas are not as thoroughly treated as others

A considerable improvement was made by Nabyea, both through the study of the vascular anatomy of several mammals and by presenting a more thorough description accompanied by semi schematic drawings and photos. However Nabeya does not present the general arrangement or a detailed description of the capillary regions, nor does he describe the apical and basal vascular areas.

Smith presented the vascular anatomy of the capillary regions in the guinea pig, the cat, and man. The instructive photographic material is accompanied by simple schematic drawings. The basal end and apical parts, however are not described. Smith's description of "a separate, narrow rolled network of vessels" in the spiral prominence in man is not adequate. The appearance of the vessel is complicated in the basal turn by the Y formed or looping connections of the supplying and draining vessels. Smith further erroneously considers each radiating arteriole to supply the stria vascularis and the vessel of the spiral prominence in the guinea pig In the present investigation, only about 15 % of the total ramifications from the radiating arterioles were found to supply the stria vascularis and a similar number the vessel of the spiral prominence. Smith describes sparse ramifications of the arterio-venous anastomoses externally to the stria vascularis. The present investigation demonstrated a considerable ramification of the anastomoses which constituted the majority of the vessels in the external wall in the basal turn.

A comparison of the present investigation with the work of Charachon is difficult since he almost completely omits a description of the venous system. His description of the arterial and capillary systems is similar to that of the present study Of the capillary regions, only the external wall was treated in detail. The photographic material and the schematic drawings are less instructive than those of Smith and of Scuderi & del Bo Wüstenfeld & Kühnert (1964) present a short description of the vascular anatomy of the guinea pig cochlea and present material largely covered by previous works. Levin's (1964) paper dealing with the vascular anatomy of the human cochlea contains a number of significant deviations from the present and previous investigations.

The findings of the present investigation are in good agreement with *in vivo*

observations of the vascular anatomy of the external wall of the guinea pig cochlea. Usually however the *in vivo* studies do not emphasize anatomical findings and only give general remarks on this subject.

Similarities and differences between the guinea pig and man

As is well known, the guinea pig cochlea has 4 turns and a relatively slender conical form while the human cochlea has 2 ³/₄ turns and a much greater diameter in the basal turn than in the others

The vascular arrangement is similar in the guinea pig and man (Fig. 84) The main arterial supply in both consists of an artery running spirally around the modiolus and radiating arterioles supplying the external wall and the spiral lamina. The capillary regions also largely resemble one another in the two species. All the spiral capillary vessels in the guinea pig cochlea can be identified in man, except the vessel of the scala vestibuli. The simplification toward the apex and the special arrangement at the basal end are also principally similar

A difference is found in the distribution of the vestibulo-cochlear artery which supplies a larger proportion of the base in man The appearance of the radiating arterioles in the modiolus is also different. In the guinea pig there are structures resembling spring-coils consisting of very tightly wound arterioles. In man the arterioles run markedly serpentine but do not appear as spring coils. The vessel of the basilar membrane, located under the tunnel of Corti, is more continuous in man than in the guinea pig where discontinuities are quite numerous, particularly apically On the other hand, the vessel of the tympanic lip seems to be better defined in the guinea pig than in man In addition, the stria vascularis is better developed at the basal end in the guinea pig than in the human cochlea The course and distribution of the veins in the modiolus is also different. In the guinea pig there is only one vein running spirally around the modiolus. In man this vein ramifies to form two large veins with frequent variations in the course and distribution of their ramifications.

The description of the vascular anatomy of the cochlea in other mammals is in good accordance with the one found in the guinea pig and man (Table I)

Apex-base

The greatest difference between the apex and the base is the increasing simplification of the vasculature in all areas apically The dense complicated vascular arrangement in the base is replaced by sparsely distributed vessels, although the general arrangement is maintained to the apex. The simplification, however is particularly marked in the two vascular structures discussed below

The breadth of the *stria vascularis* decreases considerably apically Of the dense vascular meshwork in the basal turn, only one or two vessels running spirally for short distances are left in the apical turn in both the guinea pig and man. Further there is a marked reduction of the *arterio-venous anastomoses* externally to the stria vascularis. These constitute the majority of vessels in the external wall basally particularly in the guinea pig. Apically the branches

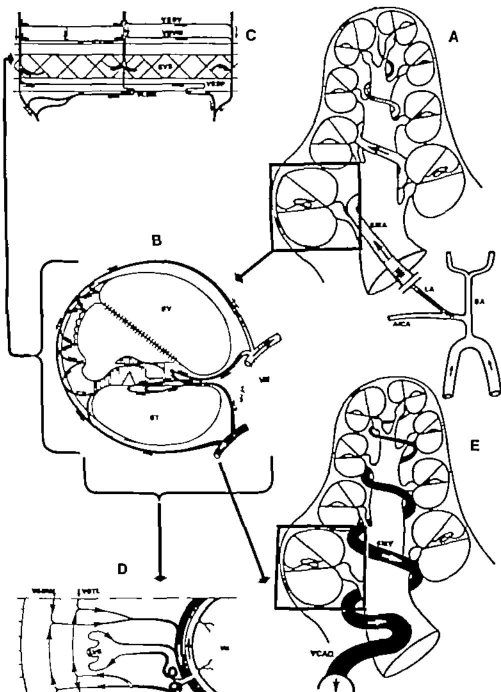


Fig 84 Composite schematic view f the cochlear vasculature with arrows indicating the direction of blood flow (veins solid, arteries unfilled).

A. *M jor arteries* VA = vertebral artery BA = basilar artery AICA = anterior inferior cerebellar artery LA = labyrinthine artery SMA = spiral modiolar artery

B. *Radial section f   half turn* (framed in A and E) SV = scala vestibuli, SM = scala media, ST = scala tympani, N VIII = acoustic nerve. Crossed-hatched areas are vascular

C. *Radial section f the external wall.* VSSV = vessel of the scala vestibuli, VSVM = vessel at the vestibular membrane, SVS = stria vascularis, VSSP = vessel of the spiral prominence, VLBM = essels the basilar membrane.

D *Transverse section f the piral lamina* VSBM = vessel of the basilar membrane, VSTL = vessel of the tympanic lip, LVS = limbus vessels, N VIII = acoustic nerve.

E. *M jor veins* SMV = spiral modiolar vein, VCAQ = ea of the cochlear aqueduct.

from the radiating arterioles supply the spiral capillary systems before reaching the venous system. The rapid flow in these anastomoses has been observed *in vivo* and was increased experimentally by anoxia, increased CO_2-tension pressor agents, acoustic stimuli and moderate hypothermia (Perlman & Kimura, 1955 a, b 1962 Perlman et al., 1959 a, b 1963 Nomura, 1961 Tsunoo & Perlman, 1964 1965) Weille (1955) considers the anastomoses to be of importance for the maintenance of a constant intracochlear temperature. The arterio-venous anastomoses thus may allow adjustment to circulation changes in the basal turn by shunting the blood past the capillaries in the stria vascularis and the other spirally running capillary systems.

However the apparently increasing sparseness apically of the vasculature has to be related to the decreasing volume of the turns. When this is done (see External Wall Comment) it appears that the actual apical simplification of the vasculature is not so pronounced The most obvious change is then the decrease in the absolute number of arterio-venous anastomoses. This change probably admits fewer possibilities for adjustment to circulation variations apically than basally

Not only the apical parts demonstrated a simplification of the vascular arrangement. This was also found at the *basal end* of the cochlea. Not all types of vessels are similarly affected by the simplification here. It is particularly the stria vascularis which is found to be reduced, in man often to the extent of being replaced by one single vessel

The basal end is also interesting in being supplied and drained from two directions. Arterioles run obliquely over the scala vestibuli and anastomose with arterioles coming from the vestibulum. An interesting region not previously described appears in the external wall between the windows where the arterioles meet. In man the arterioles from both directions derive from the vestibular branch of the vestibulo-cochlear artery On its way to the vestibulum, radiating arterioles are given off over the scala vestibuli. Other radiating arterioles turn around the extreme basal end of the spiral lamina and reverse their direction toward the scala vestibuli. A similar arrangement is found with the venous system. Branches from the posterior vestibular vein accompany the radiating arterioles around the extreme basal end of the spiral lamina. They then connect with vessels in the external wall particularly the stria vascularis and the vessel of the spiral prominence

As demonstrated (Bredberg, 1968), the development of the cochlea begins somewhat apically from the basal end and progresses simultaneously in both directions. This observation may account for the unique vascular arrangement at the basal end

Intralabyrinthine fluids

Perilymph

The vessels surrounding the perilymphatic spaces in the scala vestibuli and the scala tympani are among the various sources suggested as the origin of perilymph. Perilymph has thus been considered to derive at least partly from the vessels in the *scala vestibuli* as an ultrafiltrate of plasma (Eichler 1892, Wittmaack, 1936, Mygind, 1945, 1952, Altmann & Waltner 1950, Graf & Poretti, 1950, Kley 1951, Seymour 1954, Naftalin & Harrison, 1958, Jako et al., 1959, Schreiner 1961, Maggio 1966, Schindler & Schnieder 1966). More specifically the vessel at the vestibular membrane has been suggested as this source (Mygind, 1948, Rüedi, 1951).

Since the circulation in the external wall of the *scala tympani* is venous, the collecting venules have been supposed to absorb perilymph (Wittmaack, 1936, Arnvig 1951, Kley 1951, Svane-Knudsen 1958, Kirikae et al. 1961, Rauch, 1964). Experiments with injected material have indicated that it is particularly the spirally running venules at the basilar membrane which take part in the fluid exchange (Altmann & Waltner 1947, Svane-Knudsen, 1958). Perilymph secretion from both the scala vestibuli and the scala tympani has also been proposed (Eichler 1892, Naftalin & Harrison, 1958, Koburg & Plester 1962, Rauch, 1968).

Recently it has been demonstrated that there is a particular tissue resembling the plexus chorioideus in the region of the "*vascular spring-coils*" *in the modiolus*. This tissue may have a secretory function and take part in the formation of perilymph or its prestages (Balogh & Koburg 1965, Müsebeck, 1965 a, b, Meyer zum Gottesberge & Koburg, 1968). The "vascular spring-coils" have also been considered to have a blood pressure regulating function (Schwalbe, 1887, Mygind, 1952) and may diminish the pulse wave (Levin, 1964). They are surrounded by perivascular fluid spaces which have been proposed to regulate their caliber (Mygind, 1952).

A transfer of perilymph from cerebrospinal fluid by *perineural* or *perivascular spaces* has been suggested (Kley 1951) but an absorption may also take place along these routes (Altmann & Waltner 1950, Kirikae et al., 1961).

On the basis of the findings in the present investigation the following comments can be made. In the *scala vestibuli* there is a capillary net and readily identifiable spiral vessels: the vessel of the scala vestibuli (guinea pig) and the vessel at the vestibular membrane (guinea pig and man). These vessels have been comparatively little noticed by previous authors. They are supplied by a large proportion of the radiating arterioles (guinea pig and man) and by collecting venules (man). These findings clearly indicate that there are definite possibilities for perilymph formation in the wall of the scala vestibuli and in particular in the region close to the attachment of the vestibular membrane.

In the *scala tympani* the circulation in the external wall is completely venous. This is in good agreement with the previously suggested absorption of perilymph

here. It is probable that the venules at the basilar membrane have a central role in this absorption

The *"vascular spring-coils in the modiolus* consist of tightly coiled parts of the radiating arterioles. They are surrounded by circular smooth muscle cells and a longitudinal "elastica interna" internally (Schwalbe, 1887) or by sparse smooth muscle cells (Smith 1951) In man these vessels were considered to show about the same characteristics as those of the capillaries as their wall is thin, composed of a single endothelial tunic with very scarce smooth muscle cells and without elastic elements (Scuderi & del Bo 1952) The vessels are surrounded by abundant autonomic nerve fibers (Müsebeck & Mootz, 1966 Terayama et al., 1966)

The common type of vascular adjustment for controlling downstream pressures is produced by a narrowing of the vascular diameter rather than by changing vascular length, simply because the smooth muscles in most precapillary resistance vessels (arterioles) are nearly circular in arrangement. The curious and specific arrangement of the "vascular spring-coils" speaks in favor of a particular and specific function The existence of innervated smooth muscles might indicate a regulating function, active or passive, of events in more peripheral capillary regions.

Endolymph

The *stria vascularis* has long drawn attention as the presumed source of endolymph, of the oxygen supply to the organ of Corti, and of the generation and maintenance of the resting DC potential and the endocochlear potential. It is particularly the histological appearance and the high metabolic activity which support the hypothesis that it, at least to some extent, takes part in the formation of endolymph (Retzius, 1882 Shambaugh, 1906 Guild, 1927 Fieandt & Saxén 1937 a, Engström et al., 1955 Smith, 1957 Spoendlin, 1957 Rauch, 1963 Nakai, 1965) According to Borghesan (1957 1967) the source of "cristalloids" is probably in the stria vascularis and of "plasma" in the spiral prominence. Some authors have proposed that both secretion and absorption may take place in the stria vascularis (Mygind, 1952 Naftalin & Harrison, 1958 Rauch, 1963 Nakai, 1965 Meyer zum Gottesberge et al., 1965; Rauch & Ruska, 1965 Maggio, 1966 Kikuchi & Hilding, 1966 Hinojosa & Rodriguez Echandia, 1966) The particularly high mitochondrial content in the stria vascularis indicates a high rate of oxidative metabolism and is interpreted as supporting the hypothesis of endolymph formation here (Engström et al 1955 Chou & Rodgers, 1962 Nakai, 1965 Rauch & Ruska, 1965 Hinojosa & Rodriguez Echandia, 1966 Matschinsky & Thalmann, 1967 Nakai & Hilding, 1968)

Opinions have been divergent concerning the function of the *spiral prominence* A secretory function in the adult (Borghesan, 1957 1967) or in the embryo (Cimino & Grisanti, 1967) has been proposed. A resorptive function has been suggested by Yamamoto & Nakai (1964) and both secretion and absorption by Kikuchi & Hilding (1966)

The *external spiral sulcus* is situated immediately below the spiral prominence. This region has been considered to have a secretory function (Shambaugh, 1909 Borghesan, 1965 a, b Lawrence, 1965) but has particularly been associated with absorption (Fieandt & Saxén, 1937 b, Altmann & Waltner 1950 Saxén, 1951 Arnvig, 1951 Kley 1951 Mygind, 1952 Seymour 1954 Yamamoto & Nakai, 1964)

The *spiral limbus* has been little discussed in the formation and absorption of endolymph. An absorbing function has been suggested (Altmann & Waltner 1947 1950 Borghesan, 1957 1967 Cimino & Grisanti, 1967) The experiments of Voldrich (1967) however indicate that this region may take part in the formation of endolymph. The early and rich vascularization of the spiral limbus, found already in the embryo was emphasized by Cimino & Grisanti (1967)

The vascular supply to the walls of the scala media make it the most vascularized of the scalas. The formation and absorption of endolymph (total volume 3—5 mm^3) and the metabolic processes in the scala media thus apparently require a larger blood supply than that demanded by functions associated with perilymph (total volume 12—16 mm^3)

The present investigation cannot answer the difficult question of which regions are responsible for formation and absorption of endolymph in the scala media. From the vascular viewpoint the following findings may be of interest. The regions which probably are involved in the formation and absorption of endolymph are the stria vascularis, the spiral prominence, the external spiral sulcus and the spiral limbus.

The *stria vascularis* is supplied and drained by comparatively few but large radiating arterioles and collecting venules. It is a separate vascular system connecting only with these arterioles and venules. The breadth of the stria vascularis decreases markedly apically but the length/tissue area of the capillaries is similar in the three basal turns in spite of the seemingly increasing sparseness toward the apex. It is furthermore difficult to inject with contrast which gives the impression that the contrast is predominantly shunted over to the venules through the arterio-venous anastomoses exernally to the stria vascularis.

Similarly to the stria vascularis, the *vessel of the spiral prominence* only connects with few but large radiating arterioles in the scala vestibuli and collecting venules in the scala tympani. The angles at the ramifications of the arterioles and venules to the vessel of the spiral prominence are nearly 90 degrees. There are no vascular connections with the stria vascularis. These facts may indicate separate and specific functions for the stria vascularis and the vessel of the spiral prominence.

The present investigation demonstrated that the venules in the region of the *external spiral sulcus* assume a complicated appearance, making loops before turning around the attachment of the basilar membrane. Recent studies with electron microscopy (Engström, 1968) have shown long extensions from the cells in the external spiral sulcus to adjacent blood vessels, which is in good agreement with the proposed absorbing function of the venules in this region.

In comparison with the stria vascularis, the *spiral limbus* has been very little

1967) This was interpreted as supporting the hypothesis that sodium, potassium, and fluid may be actively transported in the region of the organ of Corti

Data have also been obtained from histopathological investigation of the human cochlear vessels. Schuknecht & Igarashi (1964) found stria degeneration without simultaneous changes in the organ of Corti.

The accumulated evidence thus suggests that the vessel of the basilar membrane and the vessel of the tympanic lip are of definite importance for the maintenance of normal function in the organ of Corti and probably also for the embryological development of this organ In the discussion of the oxygen supply to the organ of Corti it is surprising how seldom other vessels than the stria vascularis have been taken into account. However the limbus vessels, the vessel of the basilar membrane and the vessel of the tympanic lip are all situated closer to the organ of Corti than the stria vascularis Rauch (1966 1968) has further suggested that the organ of Corti may be supplied by a perineural route.

In the present investigation it was demonstrated that the vessel of the basilar mebrane is supplied by 25 % and the vessel of the tympanic lip by 75 % of the radiating vessels in the spiral lamina. These figures may indicate that the latter vessel plays a relatively larger role. The capillary region of the spiral limbus is one of the few regions which have not been discussed at all in this problem, even though it should be emphasized that it is highly vascularized similarly to the stria vascularis.

Clinical aspects

Attempts are constantly made to explain clinical hearing disorders on the basis of cochlear vascular pathology Many of these suggestions are without adequate morphological and physiological foundation. Among other reasons, this has been due to a definite lack of detailed information on the vascular anatomy

It is widely suggested today that the cochlear vessels may play an important role in such pathological conditions as congenital and hereditary deafness, presbycusis, sudden deafness, viral labyrinthitis and Ménière's disease. It was not within the scope of the present investigation to attempt to explain these disorders on the basis of the vascular anatomy However some aspects will be presented on the basis of the findings in the present investigation

A. General remarks on circulation disturbances

Since rich anastomosing possibilities exist in a spiral direction, all vascularized parts of the cochlea probably have a more or less constant circulation even in event of small peripheral flow disturbances. This suggests that higher degrees of perceptive hearing impairments of vascular origin must either be due to extensive vascular injuries peripherally in the capillary regions, or due to injuries in the larger vessels in the modiolus or in the inner acoustic meatus. A definite interruption of the circulation in the end arteries, the labyrinthine or the common cochlear arteries must result in deleterious consequences (Kimura & Perlman, 1958) On the other hand, interruption of the circulation in the

vestibulo-cochlear or spiral modiolar arteries need not lead to serious impairment since these vessels in man anastomose with one another in the basal turn. Individuals in which the spiral modiolar artery is missing and replaced by the cochlear branch of the vestibulo-cochlear artery may be more vulnerable to vascular disorders in the cochlea.

The high frequency of high tone loss of different etiology suggests that the most vulnerable parts of the cochlea are situated within the areas supplied by the vestibulo-cochlear artery and in particular the vestibular branch.

Small injuries peripherally i.e. in single radiating arterioles or collecting venules will probably not result in a clinically detectable change of hearing due to good anastomosing possibilities in a spiral direction. In the human cochlea there are also relatively good alternative pathways for the blood in case of circulation interruption in either of the spiral veins. However an interruption of the common modiolar vein or the vein of the cochlear aqueduct must have a very severe effect upon cochlear circulation. In the guinea pig arterial obstruction of the labyrinthine artery led to much more severe and rapid damage than venous obstruction of the vein of the cochlear aqueduct (Kimura & Perlman, 1956, 1958 Perlman & Kimura, 1957)

B. *Auditory function in diabetics*

Diabetes mellitus is a logical choice for investigation of a common disease which is known to be accompanied by vascular changes. It is well known that diabetes mellitus, particularly in young individuals, gradually leads to diabetic angiopathy These changes can be demonstrated in most organs and have also been observed in the cochlea (Jörgensen, 1962 Costa, 1967) Widespread vascular changes would be expected to influence even cochlear vessels with a resulting perceptive hearing loss. Such hearing impairments have previously often been demonstrated in diabetics by audiological examinations (Camisasca, 1950 Vigi, 1950 Marullo 1950 Borsuk et al., 1956 Ancona, 1956 Profazio & Baravelli, 1959 Jörgensen & Buch, 1961 Dietzel, 1964)

Recently a thorough investigation of young diabetics surprisingly revealed that the auditory function was well preserved when compared to a corresponding non-diabetic material (Axelsson & Fagerberg, 1968) This then can only be explained by a patchy distribution of the diabetic changes in the cochlear vessels where the rich vascular supply peripherally may leave many anastomosing pathways open

C. *Noise induced hearing loss*

It has been demonstrated that noise decreases oxygen tension in the scala media (Misrahy et al 1958 Koide et al., 1960) It has also been demonstrated that the cochlea is more vulnerable to noise during simultaneous lack of oxygen (Tonndorf et al 1955 Beck & Beickert, 1958 Koide et al., 1960) As is well known, different forms of noise and also several other causes of injury often result in an audiometric high tone loss. The basal turn of the cochlea thus would appear

to be most vulnerable. This has been confirmed recently by Bredberg (1968) who, however could also demonstrate a rather constant occurrence of apical lesions. It has further been demonstrated that the oxygen consumption of the stria vascularis is greatest in the base (Koide et al., 1964 Meyer zum Gottesberge et al., 1965 Rauch, 1966 Mizukoshi & Daly 1967) According to Falbe-Hansen & Thomsen (1963) the ability to carry out anaerobic metabolism is poorest developed in the base and the organ of Corti is most susceptible to "all kinds of actions"

It would thus appear that the reason for the common injuries due to noise in the basal turn can be the high oxygen consumption and the poor anaerobic metabolism (Conti & Borgo 1964) From the vascular anatomical point of view there is no apparent reason why the basal turn should be more vulnerable than the others, regardless of where the organ of Corti gets its oxygen supply

D *Otosclerosis*

The vascular anatomy of the basal end corresponding to the region of the windows and the promunturium is of particular interest with regard to otosclerosis. The presence of perceptive hearing loss in otosclerosis has been questioned and has been demonstrated to be of the same frequency as in a general population (Glorig & Gallo 1962) However most authors consider that the conductive hearing loss may be accompanied by a perceptive hearing impairment (Carhart, 1966) This has been ascribed in general to cochlear involvement. Many findings indicate the central role of the cochlear vessels in these cases and the following explanations for the perceptive deafness have been suggested:

1 Pathological connections between the vessels of the membranous cochlea with those in the otosclerotic foci (Rüedi, 1965 Altmann et al., 1966 Rüedi & Spoendlin, 1966 Linthicum, 1966)

2 Atrophic changes in the spiral ligament due to nearby otosclerosis of the cochlear capsula (Benitez & Schuknecht, 1962 Schuknecht & Igarashi 1964 Schuknecht & Gross, 1966 Lindsay & Beal 1966 Nager 1966)

3 Changes in the stria vascularis caused by the two above-mentioned processes with resulting secondary changes in the endolymph (Rüedi, 1964 1965 Altmann et al., 1966)

4 Chemical alterations of the intralabyrinthine fluids by substances released from the otosclerotic process (Rüedi, 1965 1966 Schindler et al., 1965 Altmann et al., 1966 Rüedi & Spoendlin, 1966)

5 Direct ingrowth of the otosclerotic process in the scala tympani (Nager & Fraser 1938 Nylén, 1949 Rüedi, 1961 1962 1964)

6 Vascular stasis by the influence of the otosclerotic process on nearby vessels (Rüedi, 1968)

The following findings of the present investigation are of interest concerning these problems. The basal end is comparatively poorly vascularized, especially the stria vascularis The very nearby vessels to the oval window are the radiating arterioles running from two directions and anastomosing in this region. An

extension of the disease or the influence of stapes surgery on these arterioles may probably affect the blood supply Furthermore, in more extensive cases of cochlear otosclerosis the possibility exists that the larger arterioles and veins might be affected.

Concluding remarks

On the basis of the present investigation the following research areas seem to deserve the most emphasis in the future:

a. More post mortem investigations of the vascular anatomy with the present and other methods in cases with known hearing disorders and correlation to audiological examinations.

b. Examination of all vascular regions in the cochlea with the aid of electron microscopy for investigation of the vascular walls. These investigations may give important information on the role of the different vessels in the formation and absorption of the intralabyrinthine fluids.

c. Further development of the technique for *in vivo* observation of cochlear vessels under normal and induced pathological conditions.

D SUMMARY AND CONCLUSIONS

1 A survey of the literature on the vascular anatomy of the guinea pig and human cochlea is presented as well as a survey of the methods adopted for the visualization of the cochlear vessels. The survey demonstrates that the subject has received much attention There are, however several conflicting points both concerning the larger vessels and the capillary regions. The apical and basal ends of the cochlea have been comparatively little studied Many of the divergent opinions might have been due to nomenclature differences.

2. The present investigation deals with the vascular anatomy of the guinea pig and man. The cochlear vessels were visualized by a technique similar to previously used methods. The method includes the following steps injection of contrast medium (Berlin blue) decalcification, microdissection of all parts of the membranous cochlea under the stereo-microscope and documentation of the findings with the aid of the photo-microscope The different steps in the preparation have been shortened and the time-consuming embedding step has been eliminated. Whole cochleas as well as dissected-out pieces have been stored in glycerin, a medium in which the elasticity of the membranous cochlea is well preserved and further preparation is possible

The description of the vascular anatomy in the cochlea has been separated into four regions the modiolus, the spiral lamina, the external wall and the basal end. The larger vessels and the capillary regions in these areas are described. Particular emphasis is placed upon detailed descriptions of all capillary regions, especially the hitherto largely ignored apical parts and the basal end. The findings are presented as photomicrographs of whole cochleas and dissected-out pieces. The vascular arrangement of the different areas is presented as simple schematic drawings. Previously unavailable measurements of vascular dimensions are given Comparisons are made between the findings in the guinea pig and man and between the findings in basal and apical parts of the cochlea In general the commonly used vascular nomenclature has been employed Previously undescribed vessels have been named, and more descriptive and specific names have been suggested for certain known vessels.

3 The vascular anatomy of the cochlea in the guinea pig and man is principally similar particularly in the capillary regions. The arterial blood supply to the cochlea is maintained by an artery running spirally around the modiolus (Fig 84 A) Arterioles leave the artery running centrifugally and radiate both over the scala vestibuli and over the spiral lamina, ramifying several times. The spiral capillary systems in the external wall and in the spiral lamina are drained by centripetally radiating collecting venules which empty into one or two veins running spirally around the modiolus. In man the veins take on a much more complicated and varied appearance than in the guinea pig.

In the external wall the arrangement is mainly apico-basal over the radiating arterioles in the scala vestibuli and the collecting venules in the scala tympani which connect with one another by arterio-venous anastomoses (Fig 84 B, C) In the basal turn these anastomoses are frequent, creating possibilities for

shunting the blood past the capillary regions. These spiral capillary systems run at right angles to the arterioles and venules. This suggests that the blood flow in the spiral vessels is much slower than in the radiating arterioles and the collecting venules. This has also been confirmed by direct observation.

In the scala vestibuli there are one or two spiral capillary vessels and a sparse capillary net, all lying close to the attachment of the vestibular membrane. In the scala media there is a spirally running dense vascular net, the stria vascularis, *and a spirally running capillary vessel in the spiral prominence* Both are supplied and drained by comparatively few but large vessels. The collecting venules in the scala tympani form a spiral vessel below the attachment of the basilar membrane

In the spiral lamina the arrangement is arcadic since the radiating arterioles and collecting venules derive from the same central direction and connect to the spiral vessels at right angles (Fig 84 B D) These arterioles and venules constitute *the pillars of the arcades, and the vessel of the basilar membrane, the vessel* of the tympanic lip and the limbus vessels constitute the bow of the arcades.

There are additional capillary networks in the spiral ganglion, the acoustic nerve, and in the modiolus wall which, however lack the same regular pattern.

The basal end is supplied by branches coming from two directions, i.e. arterioles radiating over the scala vestibuli anastomoses with arterioles which turn around the basal end of the spiral lamina and reverse their direction apically (Fig. 78) An anastomosing net is formed where the arterioles connect. The arterioles are accompanied around the basal end by venules which form a capillary net at the extreme basal end and connect with vessels in the external wall.

Apical *parts of the cochlea are distinguished by a marked simplification of* the vascular arrangement. However this simplification is not so apparent when related to the correspondingly decreasing volume.

Due to the pattern of ramification, the vascular system of the cochlea is segmentally arranged, resulting in good anastomosing possibilities in a spiral direction. Almost all parts of the cochlea are richly supplied with vessels in both the guinea pig and man. The vestibular and tectorial membranes and the peripheral portion of the basilar membrane in both species are avascular In the present investigation occasional connections between the vessels of the spiral lamina and those in the external wall have been demonstrated in man. The vascular systems of these regions are otherwise completely separated from one another

Suggestions are made concerning the possible role of the cochlear vessels in cochlear physiology e.g in the secretion and absorption of intralabyrinthine fluids and in the oxygen supply to the organ of Corti. Suggestions are also made regarding *the possible role of the vessels in certain clinical disorders of the* cochlea.

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The present investigation was carried out in the Ear Nose and Throat Department, Sahlgrenska Hospital University of Göteborg The animal experiments were performed in the Department of Histology and the human cadaver preparations in the Department of Pathology

The author would like to express his gratitude to Professor Gösta Herberts, head of the ENT Department, for his kind supervision stimulating discussions and constant encouragement. The author also wishes to express his debt to Professor Hans Engström for providing the original inspiration for this work for the helpful suggestions from his extensive knowledge of the inner ear and for much material aid Associate professor Olle Hallén s tireless criticism and encouragement have been of great help during the entire project Associate professor Hans Röckert contributed to valuable discussions of the microangiographic techniques as well as the later phases of the work Professor Bo-Eric Ingelmark is gratefully acknowledged for all his help with nomenclature problems. Associate professor Per Ingvar Brånemark and Dr Orozimbo Costa provided expert advice on angiologic problems.

The author wishes also to express his appreciation to Docent Uno Zachrisson and Fil Kand. Ulf Runze for their generous help with the statistical treatment of the results. Mrs. Inga Britt Christoffersson and Mrs. Ulla Larsson offered excellent technical assistance during the animal experiments Mr Georg Borgelind and Mr Uno Glans offered the same skillful assistance in the human preparations. To Anton Andersson Research Engineer I wish to express my thanks for helpful technical cooperation during the entire work The talented artistic assistance of Miss Maria Huhta deserves credit for the quality of the schematic drawings. The author wishes to thank Mr Allen Johnson, B S., for constructive discussions and correction of the English text.

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Zusammenfassung und Schlussatz

1 Die Literatur der Gefässanatomie der Cochlea, sowie der bei der Darstellung der Cochleagefässe von Meerschweinchen und Homo angewandten Methoden wird zusammengefasst. Es geht aus diesem Überblick hervor dass diese Fragen grosse Beachtung gefunden haben. Es bestehen doch mehrere gegensätzliche Auffassungen sowohl im Hinblick auf die grösseren Gefässe, als auch auf die Kapillargebiete Sowohl die apikalen als auch die basalen Abschnitte der Cochlea sind verhältnismässig wenig untersucht worden Mehrere der gegensätzlichen Auffassungen können auf unterschiedliche Nomenklatur zurückgeführt werden

2. Die vorliegende Untersuchung beschäftigt sich mit der Gefässanatomie des Meerschweinchens und des Menschen. Die Cochleagefässe wurden durch eine, einer früher angewandten Methode ähnlichen Technik dargestellt. Die Methode besteht aus folgenden Momenten Injektion von Kontrastmittel (Berliner Blau) Entkalkung, Mikropräparation sämtlicher Abschnitte der membranösen Cochlea unter dem Stereomikroskop, sowie Darstellung der Befunde mit Hilfe des Photomikroskopes. Die verschiedenen Momente des Verfahrens sind abgekürzt worden, und das zeitraubende Einbettungsmoment wurde ausgeschaltet. Sowohl ganze als auch präparierte Teile der Cochlea wurden in Glycerin verwahrt, einer Substanz, die die Elastizität der membranösen Cochlea gut erhält und damit weitere Präparationen ermöglicht.

Die Beschreibung der Gefässanatomie der Cochlea wurde in vier Abschnitte aufgeteilt· der Modiolus, die Lamina spiralis, die Aussenwand und die basale Aussenschleife. Sowohl die grösseren Gefässe, als auch die Kapillargebiete in diesen Abschnitten wurden beschrieben. Besondere Aufmerksamkeit wurde der ausführlichen Beschreibung aller Kapillargebiete gewidmet, besonders der bisher im allgemeinen wenig beachteten apikalen Abschnitte und der basalen Aussenschleife. Die Befunde wurden als Mikrophotographien von ganzen oder präparierten Cochleateilen präsentiert. Die grundsätzlichen Gefässanordnungen der verschiedenen Abschnitte wurden durch einfache schematische Darstellungen erklärt. Bisher unzugängliche Werte der Gefässausdehnung und masse wurden vermittelt. Vergleiche zwischen den Befunden am Meerschweinchen und am Menschen, sowie zwischen den basalen und apikalen Abschnitten der Cochlea wurden angestellt. Es wurde hauptsächlich die allgemein übliche Gefässnomenklatur angewandt. Bisher nicht beschriebene Gefässe wurden benannt. Genauere und mehr bezeichnende Namen wurden für gewisse Gefässe vorgeschlagen

3 Die Gefässanatomie der Cochlea des Meerschweinchens stimmt, besonders in den Kapillargebieten, im Wesentlichen mit der des Menschen überein Die arterielle Blutversorgung der Cochlea wird hauptsächlich durch eine, um den Modiolus verlaufende Arterie aufrechterhalten (Fig 84 A) Arteriolen gehen in zentrifugaler Richtung von ihr aus und verlaufen mit mehreren Verästelungen sowohl über die Scala vestibuli als auch über die Lamina spiralis. Die spiral verlaufenden Kapillargefässe der Aussenwand und der Lamina spiralis werden durch in zentraler Richtung verlaufende Sammel enolen drainiert, die sich in

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Menschen ohne Gefässe In der vorliegenden Arbeit konnten hin und wieder auftretende Verbindungen zwischen den Gefässen der Lamina spiralis und der Aussenwand beim Menschen nachgewiesen werden. Die Gefässysteme dieser Abschnitte sind im übrigen völlig voneinander getrennt.

Vermutungen werden angestellt teils über die mögliche Bedeutung der Cochleagefässe für die Physiologie der Cochlea, beispielsweise bei der Ausscheidung und Aufnahme von Perilymphe und Endolymphe und für die Sauerstoffversorgung des Cortischen Organs, teils über die mögliche Bedeutung der Gefässe bei einigen klinischen Störungen der Cochlea angestellt.

TABLE VIII

Present and previous nomenclature [1]

Arterial system, guinea pig and man.

1	Present nomenclature:	*Basilar artery A. basilaris*
2.	Present nomenclature:	*Anterior inferior cerebellar artery A. cerebelli inferior anterior A. cerebellare anteriore inferiore*
	Previous synonymous nomenclature	Stopford, 1915; Nabeya, 1923 Watt & McKillop, 1935; Sunderland, 1945; Scuderi & del Bo, 1952; Nager 1955 Beickert, 1962; Matson & Cretton, 1963; Levin, 1964; Bernstein & Silverstein (cat) 1966.
	Previous different nomenclature	*A. auditiva interna* Eichler 1892; Siebenmann, 1894
3	Present nomenclature:	*Labyrinthine artery, A. labyrinthi, A. labyrinthica Aa. labyrinthicae*
	Previous synonymous nomenclature:	Siebenmann, 1894 Shambaugh (embryo pig), 1903 Nabeya, 1923; Smith, 1951; Nager 1955; Kellner & Richter 1961; Beickert, 1962; Mazzoni & Giacomelli, 1962 Levin, 1964; Wüstenfeld & Köhnert, 1964 Williams, 1965
	Previous different nomenclature	*Internal auditory artery A. auditiva A. auditiva interna, Artère auditive interne Eigentliche A. auditiva interna;* Toldt, 1884 Eichler 1892 Siebenmann, 1894; Stopford, 1915 Watt & McKillop, 1935; Sunderland, 1943 Perlman & Kimura, 1955; Nager 1955; Naumann et al., 1958; Perlman et al., 1959; Bonura & de Stefani, 1961; Charachon, 1961; Mazzoni & Giacomelli, 1962; Matson & Cretton, 1963 Alford et al., 1965 Terayama, 1966.
4	Present nomenclature:	*Common cochlear artery A. cochleae communis A. cochlearis communis, Artère cochleaire commune*
	Previous synonymous nomenclature	Siebenmann, 1894; Anzi (dog, rat), 1908; Nabeya, 1923; Smith, 1951; Charachon, 1961 Levin, 1964
5	Present nomenclature	*Vestibulo-cochlear artery A. vestibulo-cochlearis Artère vestibulo-cochleaire A. cocleo-vestibolare*
	Previous synonymous nomenclature	Siebenmann, 1894; Anzi (dog, rat), 1908 Nabeya (man), 1923; Scuderi & del Bo, 1952 Kellner & Richter 1961 Charachon, 1961 Mazzoni & Giacomelli, 1962; Levin, 1964
	Previous different nomenclature	*A. vestibuli posterior* Nabeya (guinea pig), 1923 *The basal cochlear artery* Bernstein & Silverstein (cat), 1966.

[1]) The names in present nomenclature are the ones used in the present investigation in English followed by the same in Latin and by previous authors suggested synonymous names in German, French, and Italian

6. Present nomenclature:	*Spiral modiolar artery A. spiralis modioli*
Previous synonymous nomenclature	Introduced in the present investigation.
Previous different nomenclature:	*The cochlear artery A. cochleae Die Schneckenarterie A. cochlearis, A. cochleae propria* Boettcher 1887 Eichler 1892 Siebenmann, 1894; Asai (dog, rat), 1908; Nabeya, 1923 Smith, 1951 Scuderi & del Bo, 1952 Perlman & Kimura, 1955 1962 Nomann et al., 1958; Charachon, 1961; Illig, 1961; Mezighian & Giacomelli, 1962, Matson & Creston, 1963 Wüstenfeld & Kühnert, 1964; Levin, 1964 Musebeck, 1965 Müsebeck & Moorz, 1966; Lawrence, 1966 Terayama, 1966 *The major cochlear artery* Bernstein & Silverstein (cat) 1966. *A. spirale* Bonaccorsi & Sambuco, 1964

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7 Present nomenclature:	*Radiating arterioles Arteriolae radiatae*
Previous synonymous nomenclature	Smith, 1951 1954; Perlman et al., 1954; Perlman & Kimura, 1955 1962; Naumann et al., 1958 Kirikae et al., 1961; Nomura, 1961; Tonoo & Perlman, 1964; Lawrence, 1966.
Previous different nomenclature	*Radiating arteries, Artères radiaires* Shambaugh (embryo pig), 1903; Nabeya (guinea pig), 1923; Charachon, 1961 *Radiating branche* Nabeya (man), 1923 *Radiating arterial branches* Nabeya (man), 1923 *Arterioles* Asai (dog, rat), 1908 Mygind, 1948 Smith, 1954 Weille et al., 1954; Wüstenfeld & Kühnert, 1964 Lawrence, 1966 *Vasi radiali arteriosi, Arterie radiale Arterioli radiale* Scuderi & del Bo, 1952; Bonaccorsi & Sambuco, 1964 *Radial arterioles* Matson & Creston, 1963

Venous system, guinea pig and man.

1 Present nomenclature	*Vein of the cochlear aqueduct V aquaeductus cochleae Schneckenaquäductvene Vene der Schneckenwasserleitung.*
Previous synonymous nomenclature:	Hyrtl, 1845 Eichler 1892; Siebenmann, 1894; Asai (dog, rat) 1908; Tonndorf et al., 1962 Levin, 1964; Tonoo & Perlman, 1964 1965
Previous different nomenclature	*V canaliculi cochleae* Shambaugh (embryo pig) 1903; Nabeya, 1923; Smith, 1951 1954 Kellner & Richter 1961; Anson, 1963 *Inferior cochlear vein.* Perlman, 1952; Kimura & Perlman, 1956, 1958 Perlman et al., 1959 Griffith, 1961; Tonndorf et al., 1962; Schuknecht & El Seifi, 1963 *Schneckenvene* Eichler 1892. *V spiralis inferior* Illig, 1961 *V spiralis interossea.* Mygind, 1948

Guinea pig.

2. Present nomenclature	*Spiral modiolar vein V spiralis modioli*
Previous synonymous nomenclature	Schwalbe, 1887 Wüstenfeld & Kühnert, 1964
Previous different nomenclature	*V spiralis posterior* Shambaugh (embryo pig) 1903; Asai (dog, rat), 1908; Nabeya, 1923; Smith, 1951, 1954; Kimura & Perlman, 1956 Perlman & Kimura, 1957; Kirikae et al., 1961; Bonaccorsi & Sambuco, 1964; Tonoo & Perlman, 1964; Müsebeck, 1965; Musebeck & Moorz, 1966; Terayama, 1966

Man.

3 Present nomenclature: *Common modiolar vein V. modioli communis.*
Previous synonymous nomenclature: Introduced in the present investigation.
Previous different nomenclature: *V. spiralis posterior* Nabeya, 1923

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4 Present nomenclature: *Vein of the scala tympani V. scalae tympani*
Previous synonymous nomenclature: Introduced in the present investigation.
Previous different nomenclature: *Hintere Spiralvene V. spiralis posterior* Siebenmann, 1894, Nabeya, 1923; Scuderi & del Bo, 1952; Griffith, 1961 Levin, 1964; Maggio, 1966.
Spiral vein V. spiralis Arnvig, 1951; Kley 1951

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5 Present nomenclature: *Vein of the scala vestibuli, V. scalae vestibuli.*
Previous synonymous nomenclature: Introduced in the present investigation.
Previous different nomenclature: *V. spiralis anterior Vordere Spiralvene V. spirale anteriore* Siebenmann, 1894; Nabeya, 1923; Scuderi & del Bo, 1952; Griffith, 1961; Levin, 1964; Maggio, 1966.

Guinea pig and man.

6. Present nomenclature: *Vein of the round window V. fenestrae cochleae*
Previous synonymous nomenclature: Nabeya, 1923
Previous different nomenclature: *Vein of the upper margin of fenestrae cochleare* Shambaugh (embryo pig), 1903

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7 Present nomenclature: *Vein of the spiral lamina V. laminae spiralis, Spiralblattvene V. della lamina spirale*
Previous synonymous nomenclature: Siebenmann, 1894 Asai (dog, rat), 1908 Nabeya, 1923; Scuderi & del Bo, 1952 Bonaccorsi & Sambuco, 1964

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8 Present nomenclature: *Collecting venules, Venulae collectae*
Previous synonymous nomenclature: Smith (guinea pig), 1951; Seymour 1954; Weille et al., 1954; Perlman & Kimura, 1955 Naumann et al., 1958; Perlman et al., 1959; Kirikae et al., 1961 Bonaccorsi & Sambuco, 1964; Terayama, 1966; Maggio, 1966.
Previous different nomenclature: *Collecting vein* Perlman & Kimura, 1955
Venules Mygind, 1948; Smith (cat), 1954; Perlman & Kimura, 1955 Nomura, 1961 Tanoo & Perlman, 1964
Vv. radiatae Veines radiaires Nabeya, 1923; Charachon, 1961
Vasi radiali venosi Vene radiale Scuderi & del Bo, 1952.

		Capillary regions, External wall, Scala vestibuli.
1	Present nomenclature:	*Radiating arterioles See modiolu*
2.	Present nomenclature:	*Vessel of the scala vestibuli Vas scalae estibuli.*
	Previous synonymous	Introduced in the present investigation.
3	Present nomenclature	*Vessel t the vestibular membrane Vas membranae vestibularis*
	Previous synonymous nomenclature	Introduced in the present investigation.
		Scala media.
1	Present nomenclature:	*Stria vascularis.*
	Previous synonymous nomenclature:	Generally accepted.
	Previous different nomenclature:	*The vascular stria* Sa én, 1951
2.	Present nomenclature	*Vessel f the spiral prominence Vas prominentiae spiralis.*
	Previous synonymous nomenclature:	Perlman & Kimura, 1955; Wüstenfeld & Kühnert, 1964
	Previous different nomenclature:	*The piral prominence vessel, Vas prominens, Vas prominence* Hensen, 1871; Waldeyer 1872 Toldt, 1884 Saxén, 1951; Smith (guinea pig), 1951; Perlman & Kimura, 1955; Bonaccorsi & Sambuco, 1964 Terayama, 1966. *N twork f the piral prominence, The blood vessel under the spiral prominence The vessels in the spiral prominence The vessels of the spiral prominence Gefäss der Prominentia spiralis, Spiralgefäss der Prominentia spiralis, Vasi della prominenza spirale Réseau du bourrelet piral.* Asai (dog, rat), 1908; Scuderi & del Bo, 1952 Smith (man), 1954; Perlman & Kimura, 1955; Naumann et al., 1958; Charachon, 1961; Nomura, 1961
3	Present nomenclature	*Arterio-venous anastomoses Anastomoses artério-veineuses.*
	Previous synonymous nomenclature:	Perlman & Kimura, 1955; Charachon, 1961; Nomura, 1961; Irwin et al., 1964
	Previous different nomenclature	*Straight vessel in the thicker part f the spiral ligament* Smith, 1951 1954 *Arteriovenous arcades, Arteriovenösen Arkaden:* Weille et al., 1954; Smith (man), 1954 Perlman & Kimura, 1955 1957; Perlman et al., 1959; Charachon, 1961; Illig, 1961 Bonaccorsi & Sambuco, 1964; Tsunoo & Perlman, 1964 *Arcate anastomotiche arterio-venose* Agazzi, 1948; Scuderi & del Bo, 1952. *Arteriovenous shunt.* Smith, 1954 Perlman & Kimura, 1955 *Arterial venous shunt.* Matson & Creston, 1963
		Scala tympani.
1	Present nomenclature	*Collecting venules. See Modiolus.*

2.	Present nomenclature:	*Venules t the basilar membran Venulae membranae basilaris.*
	Previous synonymous nomenclature:	Introduced in the present investigation.
	Previous different nomenclature:	*A spiral vessel in the crest f the spiral ligament* Smith (man), 1954 *Vas s ala-angulare* Svane-Knudsen, 1958 *Spi alg fässe der Crist basilaris* Asai (dog, rat), 1908

Spiral lamina.

1	Present nomenclature	*The vessel f the basilar membrane Vas membr nae basilaris*
	Previous synonymous nomenclature:	Introduced in the present investigation.
	Previous different nomenclature	*Spiral vessel, Vas spiralis, Vaisseau spiral* Kölliker 1854; Gottstein, 1871 Waldeyer, 1872; Toldt, 1884; Baginsky 1886 Boettcher 1887, Schwalbe, 1887; Eschler 1892; Arnvig, 1951 Rüedi, 1951; Scuderi & del Bo, 1952; Charachon, 1961; Rauch, 1964 Bonaccorsi & Sambuco, 1964 Alford et al., 1965; Terayama, 1966; Kikuchi & Hilding, 1967 *Vessel below the tunnel f the organ of Corti, Spiralg fäss des Cortischen Tunnel* Siebenmann, 1894; Asai (dog, rat), 1908 Smith (man), 1954 *Terminal loops of vessels lying under the tunnel of Corti* Shambaugh (embryo pig), 1903 *Äusseres Spiralgefäss, Äusseres vas spi ale* Kölliker 1852 Siebenmann, 1894 *Spi al vessel beneath the tunnel f C rti.* Kikuchi & Hilding, 1967
2.	Present nomenclature:	*The vessel f the tympanic lip Vas labii tympanici*
	Previous synonymous nomenclature:	Introduced in the present investigation.
	Previous different nomenclature	*Inneres Spiralgefäss* Siebenmann, 1894; Asai (dog, rat), 1908 *Spiralg fäss des Labium tympanicum, Vaso spirale del labium timpanicum, Vas spirale labii tympanici* Siebenmann, 1894; Asai (dog, rat), 1908; Scuderi & del Bo, 1952. *Spi al vessel below the inner pilla* Terayama, 1966. *Vas spirale internum.* Deiters, 1860.
3	Present nomenclature:	*Limbus vessels Vasa limbi.*
	Previous synonymous nomenclature	Introduced in the present investigation.
	Previous different nomenclature	*Vessels f the limbus* Smith (guinea pig), 1951 *Rete capillare spirale della cresta spirale* Scuderi & del Bo, 1952.

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S U P P L E M E N T U M 245

CONGENITAL FIXATION OF THE STAPES FOOTPLATE

BURNS C. STEELE

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BURNS C. STEELE

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INTRODUCTION

Today the otologic surgeon may attack problems of conductive deafness with confidence because of the wealth of knowledge investigators have compiled in the last decade. The mysteries of the middle ear have been unveiled well documented and beautifully reproduced on film (1 2) One of the mysteries which had gone unrecognized in the premobilization days is the entity congenital fixation of the stapes footplate which will be discussed in this paper Thus, with this additional knowledge and a commensurate understanding of embryology the possibility of aiding the patient is greatly enhanced.

The purpose of this thesis is to discuss this recently established entity with reference to its origin, studies of literature, diagnosis, concepts of treatment and to present a long term review of the results of treatment on a series of cases. In addition, several recent cases will be presented

None of the cases presented in this paper have shown any family history of congenital footplate fixation. However the possibility does exist that some hereditary (3) factors may predispose the patient to this entity Certainly the progress that has been made in the study of genetics since the last 20 years had opened up new avenues of study and it is the hope of the author that this paper may stimulate otolaryngologists to take more of an interest in this field.

EMBRYOLOGY

The comprehension of the embryogenesis of the stapes will prepare the otologic surgeon for any abnormalities present and will enable him to employ the proper modalities in correcting this defect. Of great importance is the present concept of the double origin of the stapes. The major part of the structure arises in mesenchyme at the cranial end of the second visceral (hyoid) bar while the medial (vestibular surface) of the footplate derives from the cartilagionus otic capsule—a condensation termed the lamina stapedialis.

The stapes is the first ossicle to make its appearance in the 7 mm (4$^1/_2$ week) embryo and by the 6th week the mesenchyme primordia are formed and continuity of the ossicular chain established.

The stapes at this stage is a distinct ring-shaped, pre-cartilaginous mass, the center of which is pierced by the stapedial artery It is adjacent to the cells which will later form the lateral wall of the otic capsule The pre-cartilaginous cells eventually become the hard bony otic capsule



Fig. 1 Early stag in dev l pment f h man stapes, ill trating dual origi f stapes footplate—hyoid r second lateral ba nd cartilagi ous tl capsule Laml tapedi l deri ed from tle capsul Fetus f nl weeks (40 mm crown rump length) 45 Fig. 1 1 from the Wisconsi collection, series 148, courtesy f Dr Barry J Anso

In the embryo of 30 mm the otic capsule and the stapes are undergoing similar change to true cartilage. An exception to this is the lamina stapedialis, against which the stapes rests. This structure consists of two distinct cellular zones The inner is of mesenchymal character and lies on contact with the perichondrium of the stapedial base. The outer layer which is fibroblastic, blends medially with the loosely arranged mesenchyme which surrounds the epithelial labyrinth and in which the periotic spaces are already developing

The development of a lamina stapedialis prepares for the appearance of a future fenestra but does not represent the window itself and one may see that labyrinthine window as such never exists

In the 40 mm embryo (10 weeks) the stapes is still entirely cartilaginous but has lost its simple form and is changing from a ring shaped to a stirrup-shaped structure

By the 17th week the stapes has increased considerably in size. The base has become flattened and fits snugly in the window and is attached to the window through a stapedial ligament. In the 161 mm embryo the stapes has almost reached adult size and a center of ossification has formed in the basal portion The base becomes hollowed and acquires a narrow cavity and a fenestrated internal surface The vestibular layer remains cartilaginous. At this stage the crura have reached their definite size and have be-

come hollowed by a marrow cavity which is continuous with that of the base. In addition, the vestibular window reaches its definite size and shape and conforms to the shape of the base of the stapes.

Of greatest importance in the understanding of congenital footplate fixation is the formation of the annular ring, which enables the stapes to become a separate mobile structure, capable of responding to vibratory stimuli. Subsequent fusion of the lamina stapedialis with the otic capsule and the secondary differentiation of the laminar tissue provides the stapedial rim, the vestibular surface of the base and the basal perichondrium. Further modification of the peripheral laminar tissue leads to formation of the annular ligament of the stapes. The annular ligament, with the exception of the fissula ante fenestram, is the most inconstant structure encountered and its site in the 43 mm and 50 mm stages is represented by the peripheral remnant of the lamina stapedialis.

Of additional importance in the surgical considerations of a fixed footplate in the oval window *is the possibility of an irregular oval cleft. The* form of the oval cleft, which is thus produced between the stapedial footplate and the fenestral rim may not be uniform: anteriorly it may slant in such a way as to cause a segment of the footplate to lie on the medial or tympanic aspect of the window. Thus it may override the margin. Clinically it would indicate that an attempt to mobilize a fixed stapes would find an obstruction in the part nearest the cochlea.

ETIOLOGY

The embryological studies (5, 6, 7, 8, 9, 10) which have been presented clarify the concept as to the anatomical basis of congenital stapes fixation. Some of the factors which might cause a failure of the lamina stapedialis to differentiate and separate from the otic capsule will be discussed. An observation by Elliott (11) may be a useful aid in studying congenital ear malformations. He has presented evidence suggesting that after six months the axial presentation of the fetus into the vertex or breech depends upon labyrinthine activated kicking by the fetus. Progressive dominance of vertex presentation appears due to the increase of specific gravity for the axially floating fetus in its capsule.

Persistent mal-presentations result where damage or malformation of inner or middle ear exist. This is a convenient clinical manifestation on which to begin early investigation of congenitally acquired ear aplasias.

In the series of House (12), which is the largest number of reported cases of congenital footplate fixation, no evidence of a positive family history of this condition exists. Altman (13) also states that a positive family history is extremely rare in congenital malformations of the middle ear.

From the work of Anson and Bast (7, 8) the failure of separation

terminating in congenital fixation occurs at stages later than the 50 mm one

The effects of viruses, particularly of the rubella virus (14 15 16) have been studied as causes of deafness, however their mode of action has not been understood Richards (17) has reported several cases of rubella mixed deafness with congenital fixation of the stapes However Kelemen (18) in his investigation of the rubella effect on congenital stapes fixation found that in 25 hard of hearing persons with history of maternal rubella during the critical period, no evidence of conductive deafness existed Thus, since no exploration of the middle ear was performed, the presence of an anomaly could not be determined Connelly (16) stated that there is a 16% hazard of major congenital anomaly in live born children if maternal rubella is contracted in the first 2 months of pregnancy In addition he clarifies the effects of other viruses on the fetus as a whole during this period. Poliomyelitis will cause a higher death rate but not a higher malformation rate Mumps and chickenpox do not have any apparently harmful effect in pregnancy while measles seems to produce a higher fetal morbidity and malformations than normal but less than rubella Smallpox vaccination in pregnancy is definitely contraindicated and further exploration of the hazards to the use of live polio vaccine is indicated

The action of various chemical agents on the embryo has initiated considerably investigation (9 16, 20 21) Certainly the disastrous effects of thalidomide (22) has awakened us to the danger and not surprisingly the literature on teratology is now becoming inundated by a mass of information on the teratogenic effect of the new generation of synthetic compounds. According to Woollam (23) drugs such as antihistamines, alkaloids, antibiotics, cortisone, hormones, vitamins, have been known to have teratogenic effects.

M d Avignon and B Barr (24) have produced statistics illustrating effects of thalidomide Out of 904 children examined at the Karolinska Hospital during the pre thalidomide period 1944 to 1958 and found to be totally deaf or to have severe hearing loss, 13 had congenital atresia. During the 1959 to 1962 period, when thalidomide was available, however this type of deformity was found in 16 out of 57

According to Livingstone (25) thalidomide has produced hearing loss due to congenital stapes footplate fixation per se reported in the literature due to thalidomide However Livingston does offer a warning that some of the thalidomide babies, with apparently normal ears, may have a hearing defect

There are no reports in the literature listing radiation as a direct cause of congenital fixation of the stapes footplate However the effect of radiation on the stapes during the period of formation should be considered as a factor causing retadation or failure of the lamina stapedialis to differentiate and separate from the otic capsule. Experimental studies by Berg and Lindgren (26) consisting of radiation of the middle ear in rabbits showed



Fig 2. Phot micrograph f congenital fixation of stapes footpl t X t the union f the st pes footplate with the bo y otic capsul posteri rly Anteriorly partial nlar ligament exists with bo y fixatio f the vestibular l mella f th footplate. Hem toxy lin and eosi stain. x 60 Courtesy of The Los Angeles Foundation of Otology

that in milder reactions the mucous membrane became thickened and fibrotic The ossicles were frequently involved, resulting in fixation.

Since congenital footplate fixation is the result of disturbed genesis, the otologist should be aware of some factors involved. Recent advances in the studies of DNA and RNA synthesis have provided us with much information on the orderly process of embryogenesis. One of the problems which troubles investigators (23) at the present time is revealed by the hypothesis which states that the teratogenic agents each produce one of a number of possible patterns of malformation and that the choice of pattern depends upon the particular metabolic phase with which the teratogen interferes.

Another problem that must be solved, when endeavoring to elicit the mechanism of action of the teratogen is the number of levels at which the activity can be regarded as taking place This means that a decision must be made as to whether it is most important to investigate its effect upon the tissues at the molecular level, the level of the organelle or the level of the cell

At the first level we shall be investigating possibilities such as the inhibition of DNA synthesis at the level of the organelle We shall be studying for example whether the drug effects the lyosome, Golgi apparatus or mitoch ndria, at the cell level we shall be interested in its effects upon division of the cell

There are five possible end results of the effect of the teratogen at the cellular level it can stimulate cell division it can leave a cell unharmed, it can slightly effect the cell slowing down rate at which it divides, it can stop it dividing, it can kill it In practice, the effect of a teratogen is to prevent the cell from being in the right place, doing the right thing at the right time so that for example, two separate components, such as the lamina stapedialis and the otic capsule, which should separate and differen tiate fail to do so, causing a retardation or prevention of the formation of an annular ligament It would seem that in general the effect of teratogen is to kill cells without killing the embryo thus depriving the developing structure not only of a single cell but of all its descendents.

PATHOLOGY

Histologic studies of relatively rare specimens of temporal bones have revealed only slight degrees of difference in the amount of osseous union between the stapes footplate and the surrounding portion of the otic capsule There may be a complete osseous union between the footplate and the otic capsule, or in some sections, only fibroblasts may be present along the fenestral margin. This may indicate an attempt to form the annular ligament

In footplates with minimal ossification, the footplate may be thicker along the fenestral margin and thinner in the center In congenital foot plate fixation the entire footplate blends into the surrounding otic capsule, which may have a faint bluish color This color is often seen in other con genital middle ear deformities and has no particular significance, in addition there is no increase in vascularity which is seen in otosclerosis.

The footplate is composed of laminations found in primordial bone structure, however there may be islands in the crura and in the surrounding capsule showing an advanced bone formation This structure may consist of two distinct layers, an outer layer which is continuous with the stapes crura and an inner layer blending with the capsule Between the layers may be islands of cartilage and fibrous tissue, surrounded by cartilage

In the case of congenital footplate fixation described by Lindsay (30) he found that the inner lamella of the stapes footplate was continuous or fused with the otic capsule, and the other layer was continuous with the crura of the stapes.

This histologic picture seems to confirm an incomplete fusion of the annulus stapedialis with the lamina stapedialis and the failure of the latter to separate from the otic capsule

REVIEW OF THE LITERATURE

During the last decade we have learned that ossicular deformity with a patent meatus and normal tympanic membrane are not uncommon A diag



Fig. 3. Photomicrograph f congenital fixation of stapes footpl t showing normal ppearing ular lig me t t riorly Post riorly there is partial bony fixation. H ma-t xyll a d eosin stain. × 20 Co rtesy f The Los A geles Foundation f Otology



FIG. 4 *Photomicrograph f congenital fixati f stapes footplat* showi g complet f sion f footpl t post riorl with the bony otic capsul A teri rl l thi secti l g m t at (Hematoxyli nd eosin stal 40.) Courtesy f The Los Ang les F n ti f Otology

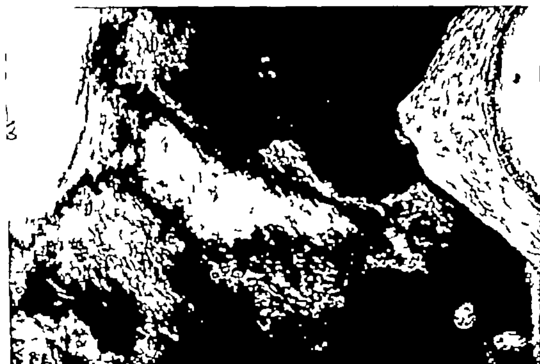


Fig 5. Phot micrograph f the posterio margi f th footplat (ight) nd the adj i ing the capsul (l ft) Not the partial formati of an ula ligament. 180 Courtesy of the Los Ang les F undatio of Ot logy

nosis may be readily anticipated and confirmed by tympanotomy Shambaugh (27) in 1952, was one of the first otologic surgeons to advocate the use of the operating microscope in middle ear surgery presented five cases of congenital stapes fixation, and cited the difference between this condition and otosclerosis. The salient points of differentiation showed that the deafness began at birth and was not progressive. No family history of otosclerosis was present in any of these cases and upon exploration of the middle ear fixation of the stapes was present without evidence of an otosclerotic focus. These cases were fenestrated and all showed hearing gain, the longest postoperative improvement being maintained after three years.

Holmgren (28) in 1957 described a case with incomplete development of the annular ligament resulting in a fixation of the stapes footplate

In 1958, House (12) described congenital stapes footplate fixation as a definite entity which could be suspected and readily differentiated from otosclerotic footplate fixation Characteristic audiograms showed a flat 45 to 55 decibel pure conductive loss and that there is no progression during adolescence ... House in reviewing a long term study of conductive deafn ... it seems logical that any child under 12 years age with n ... gs, who has a purely conductive hearing impairment, ... decibel has a congenital footplate fixation

rather than clinical otosclerosis An analysis of our operated cases of congenital footplate fixation in children confirms this hypothesis 85% of our series noted hearing loss before they were 12 years of age" The long term results of surgery on these cases will be reviewed in this paper

Hough (1 2) in his well illustrated presentation of middle ear malformations, describes a case of congenital stapes fixation with an abnormally small stapes with extremely thin crura The footplate was small and thick and blended into the surrounding otic capsule Mobilization of this footplate was successful He has described several other immature stapes with congenital footplate fixation One of them occurred in a five year old child whose hearing was restored to normal level by a partial stapedectomy

Histopathologic studies of congenital atresia, with stapes fixation, were first made by Keleman (20) in 1943 who reported an abnormal stapes, in which an annular ligament was partially replaced by bone Also, Altman (13) in 1949 reported a congenital atresia with normal stapes and an annular ligament however fixation occurred due to the presence of an osseous bridge between the head of the stapes and the fallopian canal

The first case of congenital footplate fixation with a patent meatus to be studied histologically was reported by Lindsay Sanders and Nager (30) in 1960, Sooy (31) described four cases of congenital footplate fixation These were taken from a series of 1112 operative procedures. In the case of a 12 year-old boy with a congenital footplate fixation, he states that in attempting to create a footplate by multiple circumferential performation, a sudden profuse flow of perilymph erupted on the first perforation, which rapidly filled the middle ear and the auditory canal The middle ear was filled with gelfoam and an external dressing applied Several dressings were soaked with perilymph before the flow ceased spontaneously Audiometry one month later showed a 5 dB gain, with a residual air bone gap of 60 decibels.

Cases of congenital footplate fixation have briefly been described in the literature by Henner (32) and Hajek (33) respectively however no definite treatment was described

In 1962 Escher (34) reported a bilateral congenital stapes fixation in the daughter of an otosclerotic mother whose diagnosis had been confirmed at the time of surgery In another case the son of an otosclerotic father was found to have bilateral congenital stapes fixation and malformation of the incus.

The occurrence of facial anomalies, of a less severe nature than Treacher Collins, with congenital fixation of the stapes, was described by Edwards (35) in 1964 He cites the case of a 3-year-old child with a bilateral conductive deafness. The child s mother contracted rubella before the 12th week of pregnancy Various facial anomalies were present The meati were present but narrow and normal tympanic membranes were present Exploratory tympanotomy revealed bilateral congenital fixation of the stapes footplate

Edwards states that he has performed successful total stapedect my with vein graft, polyethylene prosthe is on two cases (three ears) Th durati

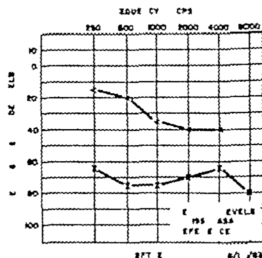


Fig. 6.

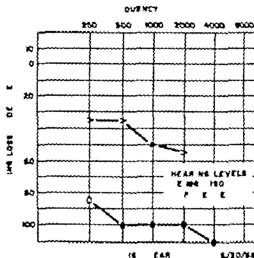


Fig. 7

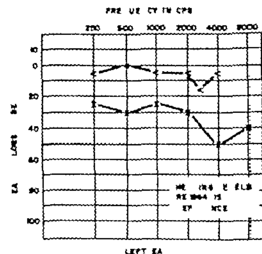


Fig. 8.

Fig. 6. Case 1 Preoperative audiogram (left ear). Air bone gap with evidence of sensorineural component

Fig. 7 Case 1 Preoperative audiogram. Air bone gap present, however sensori neural loss greater than the left ear

Fig. 8. Case 3 Preoperative audiogram showing mass tilt produced by fluid in the middle ear and superimposed upon a congenital fixation of the stapes footplat

of this hearing gain is not mentioned It is however his opinion that stapedectomy involves a considerable risk to the patient's hearing.

Ombredanne (36) whose experience has included over 100 cases of minor auditory aplasias, discusses his surgical treatment of some cases of congenital footplate fixation Whenever possible he attempts to remove the footplate and apply a thin vein graft to the oval window The stapes is then turned over in such a manner that the head is placed over the center of the vein graft The crura are then maintained under the long process of the incus. He reports a similar case as Sooy's (29) in which a very marked perilymph flow lasting three days, occurred in a case with congenital fixation of the stapes footplate This followed an attempt to free the footplate by multiple punctures. No labyrinthine or meningeal complication

followed this and after six months he reports a significant hearing gain His paper reports two cases operated upon, using the above technique and on a third case he performed a fenestration

Walsh (37) in 1965 described the case of a 25-year-old male with a history of having been deaf since birth In February 1962 a left exploratory tympanotomy which was performed revealed the stapes to be joined to the long process of the incus by a thin spicule of bone The footplate was solid, and the posterior crus was abnormally thick and wide No attempt had been made to remove the abnormal stapes In March 1962, fenestration was performed and two years later the right side was fenestrated Hearing of a very serviceable level had ben restored to this patient by fenestration and according to Walsh, attempt at stapedectomy might have been disastrous.

In 1965 T M Banham (38) reported the case of a 36-year-old white female, who complained of a unilateral deafness since childhood There was no family history of deafness, nor was there any history of middle ear infection Both meati were patent and the tympanic membranes were normal Tympanotomy revealed congenital fixation of the stapes A detailed description of the footplate was not given and stapedectomy was performed in October 1964 using the vein graft and polyethylene strut A 35 dB gain was reported for the lower frequency two weeks postoperatively however no long term results have been mentioned

So intensified has the search for evidence of stapes fixation become, as is evidenced by the investigations of Holzheuter Gregg and Clifford (39) on ancient Indian skulls. Specimens ranging from birth to over 40 years were examined Despite long internment, some burials dating to about the time of Christ showed that the skeletal details were preserved In all 417 temporal bones from 221 skulls were examined. In 19 temporal bones the stapes was in the oval window (4.5%) There was no evidence of stapes footplate fixation and no congenital anomaly of the auditory ossicles found

The presence of a congenital footplate fixation may occasionally preclude anomalies of the facial nerve A normal fallopian canal may be present, however at times a bare nerve can be seen traversing the middle ear Caparosa and Klassen (40) recently reported the presence of a bilateral congenital fixation of the stapes footplate in a 15-year-old white male In each ear the transverse portion of the facial nerve divided and passed over the stapes footplate and then united posterior to the poste ior crus Foot plates were described as being bluish in color but were very hard A small fenestra was made in the left footplate using a cutting burr the f otj lat was approximately 1 ½ to 2 mm in thi kness. A wire-f t or sthesis wa used, however no change in hearing postoperatively w rep rted

DIFFERENTIAL DIAGNOSIS

Although the other pathologic entities producing ir bone gap in the pate i meatus in an intact tympanic membrane are very important in es-

tablishing a diagnosis, this paper will limit the discussion to the entities producing fixation of the stapes footplate *per se*

Although clinical otosclerosis in a child under the age of twelve is un common it is an important entity to be differentiated from congenital foot plate fixation Family history and audiometry may aid in the diagnosis, however confirmation may only be attained by exploratory tympanotomy During adolescence a rapid progression of hearing loss in otosclerosis is a salient point of differentiation. In contrast there is no progression of hearing loss in congenital footplate fixation Often the history of deafness in con genital footplate fixation may be misleading because as the child matures the hearing deficiency may appear to be greater due to the increased demands of society in the outside world.

In congenital stapes footplate fixation, the great majority of patients first notice hearing loss before the age of twelve, they have a negative family history of deafness, they have normal physical findings and they have an audiometric graph of a flat curve between 45 and 55 decibels pure conduc tive loss.

In the child with the sequelae of a chronic otitis media with an intact tympanic membrane and a conductive loss of about 45 dB or more, a diag nosis may perhaps only be established by exploratory tympanotomy Such a problem will be presented by this writer in the case presentations.

Another condition to be considered in the differential diagnosis is fixa tion of stapes footplate as the result of an ossifying otitis media, resulting from an intra-uterine and neo-natal otitis, as described by Hemsath (41)

Fixation of the stapes footplate may occur as the result of osteogenic activity involving free cartilage rests in the annular ligament itself As a result, the bridges of cartilage in the annular ligament could become ossified in the middle decades of life such as cartilage in other areas of the body becomes ossified, thus producing fixation.

As a result of repeated attacks of chronic otitis media, a footplate fixa tion may occur due to actual arthritic changes, circumferential involvement of the annular ligament may mimic a congenital footplate fixation

The fixed malleus syndrome is an important entity to recognize and differentiate from a congenital footplate fixation It has been described to co-exist or occur as a complication of otosclerosis and it can be present with a congenital hearing loss. Goodhill (42) has described the stapedial involvement to be a lateral fixation rather than the customary medial or footplate fixation A well defined annular ring may be present which does not exist in a congenital footplate fixation Audiologically in contrast to the flat graph of congenital footplate fixation, the bone conduction curve is usu ally depressed and consistently seems to slope downward in the higher frequencies.

Osteogenesis imperfecta, a disease characterized by blue sclera, brittle bones and hearing loss, is known to be transmitted as a dominant mendelian trait Audiometrically an air bone gap is present in cases with hearing loss, grossly and histologically the lesion is identical with otosclerosis.

[illegible]

Paget's disease which is a chronic progressive condition beginning in middle or later life may involve the temporal bones, producing deafness. Deafness may be conductive mixed of sensorineural character depending upon the stage of development of the disease in the labyrinth. Histologic examination of footplate reveals new bone formation with typical mosaic pattern.

Tympanosclerosis, an entity which is the end result of chronic inflammatory middle ear disease, may produce fixation of the stapes footplate It is possible for it to occur silently with an intact tympanic membrane, thus producing a marked conductive loss. The fixation of the stapes footplate may be due to tympanosclerotic plaques which, when carefully removed, would result in mobilization of the footplate Due to ossification of the tympanosclerotic plaques in the oval window the stapes may become solidly fixed, requiring stapedectomy

In concluding discussion of the differential diagnosis of stapes ankylosis, it is important to mention the possibility of anomalies of the ossicles and windows in a patient with a patent meatus and normal tympanic membrane

TREATMENT

In congenital fixation of the stapes footplate in which the footplate is relatively thin, an attempt should be made to mobilize the footplate Mobilization may be accomplished in certain difficult cases by shattering the footplate If exploratory tympanotomy on a child reveals a congenital fixation of the stapes footplate with a complete fusion of the footplate with the surrounding otic capsule, fenestration nov-ovalis should be considered only when the child has reached young adulthood. The reason for this is that the postoperative care of the child after fenestration is much more difficult than the adult and the danger of infection is greater Until the time that fenestration can be performed, the child should be provided with a hearing aid

There is no contraindication to stapedectomy particularly if it can be performed without a drill-out. Since the incidence of sensorineural loss in fenestra nov-ovalis and stapedectomy in congenital footplates is 2% stapedectomy is the operation of choice because of the decreased incidence of infection postoperatively

Several authors (35 37) have cautioned against performing a stapedectomy because of the possibility of causing a severe sensorineural deafness. Edwards (35) states that the failure of differentiation in the annular area may also be accompanied by a variation in the anatomical position of the utricle and saccule in relation to the vestibular surface of the footplate House (43) contends the danger to these structures may be avoided by gently sucking out the perilymph and allowing these structures to collapse away from the oval window

PRESENTATION OF CASES

The case presentations consist of three children under the age of twelve years observed during the performance of 450 transcanal tympanotomies by this writer

In all of these cases a loss of hearing at an early age was the chief complaint Since two of the cases had chronic otitis media, a hearing loss due to the sequelae of this disease had to be considered in the diagnosis No family history of deafness was present in any of these cases.

Postoperatively the cases have been followed from a minimum of two months to a maximum of three years

History

Case 1 V H This 6-year-old white female was first seen on 12.0 62 the chief problem according to the mother was marked difficulty in hearing since birth A school nurse, who had advised immediate examination stated that the child talked very little There was no history of maternal rubella

Physical examination

The right tympanic membrane showed evidence of an old otitis media and consisted chiefly of a thin mobile neotympanum. The Rinne test was negative The eustachian tube was readily inflated. Nose and throat examination revealed no abnormalities Radiologic examination revealed infantile development of the mastoids Examination of the left ear revealed a large central perforation of the tympanic membrane At the time there was no discharge present in the middle ear mucosa appeared normal the ossicles were not visualized Rinne test was negative

Impression

The findings indicated a possibility of an interruption of the ossicular chain in addition to a large central perforation. Tympanoplasty with temporal fascia and canal skin graft was planned.

Surgical findings and procedure

Exploration of the left middle ear via the postauricular incision after the canal skin and temporal fascia grafts had been taken revealed limited motion of the long process of the incus. After adhesions were removed from around the stapes, it was apparent that there was a complete absence of the annular ligament and that the bony footplate blended with the surrounding otic capsule There was a slight bluish tint to the bone of the otic capsule Mobilization of the stapes was not possible in this case Malleus and incus were normal and mobile Round window and fallopian canal appeared to be normal and no evidence of an aberrant facial nerve was seen Since no further pathology was present in the middle ear of mastoid

bone the tympanoplasty was completed in the usual manner by placing the temporal fascia graft upon the deepithelialized remnant of the tym panic membrane and replacing the canal skin graft in its normal position The operative findings thus presented a diagnosis of congenital fixation of the stapes footplate.

Preoperative impression (right)

Since the audiometric findings in each ear were similar and since a congenital footplate was present in the left ear the same finding was anti cipated in the right ear In addition the possibility of ossicular fixation or interruption might be present.

This patient was referred to Dr Howard P House, who performed ex ploratory tympanotomy on 6.23 66

Surgical findings

Exploratory tympanotomy revealed an intact ossicular chain, with a nor mal mobile stapes footplate The annular ligament was complete and movement of the stapes elicited a round window reflex. Remainder of the middle ear was normal, however a faint bluish color was also noted in the otic capsule of this ear

Comment

This case has presented a unique problem in diagnosis and certainly the only solution was in the exploratory tympanotomy Similarity in the audiogram suggested similar findings. A congenital fixation of the stapes foot plate was presented in the left ear however in the right ear a normal mobile stapes was found. At the time of surgery House commented on his finding and called this an inner ear conductive deafness The possible cause according to House (43) is an obstruction in the cochlear duct. To date he has seen fifty such cases and will report on this unusual finding when histopathologic studies are available. This case illustrates two distinct findings, one of which as yet cannot be embryologically explained.

History

Case 2 H. D This 12 year-old white female was first seen on 9.24 64 Her chief complaint was loss of hearing in both ears for four years, during this period she had considerable difficulty in school

Past history

Essentially negative

Family history

Indicates a 10 ear-old sister with normal hearing and the remainder of the family app ntly has normal hearing.

Physical examination

Both auditory canals and tympanic membranes were normal eustachian tubes were readily inflated nose and throat examination was normal

Audiometric findings

The right ear reveals a flat curve of 40 dB ISO air conduction loss The left ear reveals a 3o dB air conduction loss. Bone conduction tests revealed normal cochlear function.

Impression

The presence of an air bone gap reveals the possibility of an ossicular fixation More specifically the possibility of a juvenile otosclerosis or a congenital footplate fixation.

Surgical findings and procedure

On 7 20 65 exploratory tympanotomy of the right ear revealed a fixed stapes footplate, which was markedly thickened at the periphery and thinner at the center There was no evidence of increased vascularity around the oval window No definite annular ligament was seen A stapedectomy was performed using a stainless steel prosthesis and gelfoam placed over the oval window

Postoperative results

After one year the right ear showed an average loss of 15 decibels.

Comment

This case illustrates a typical congenital fixation of the stapes footplate Since no unusual difficulty was encountered in performing the stapedectomy it can be assumed that the union with the otic capsule was not an entirely osseous one There was not an unusual flow of perilymph encountered during the removal of the stapes footplate.

History

Case 3 DeC This 6-year-old white female was first seen on 3.28 66 According to her mother she had difficulty in hearing for approximately 3 years or more She has had repeated attacks of otitis media with particular involvement of the left ear Audiometric examination at school revealed a definite loss in the left ear

Past history

Negative except for measles chickenpox and an adenotonsillectomy which was performed at the age of 3½

Physical examination

The right tympanic membrane was slightly thickened, injected and somewhat mobile The left tympanic membrane was thickened, injected, slightly bulging and immobile.

Surgical findings and procedure

On 7.25 66 exploratory tympanotomy revealed a moderate amount of thick tenacious discharge in the left middle ear This discharge was removed with an aspirator No gross abnormality of the ossicles was noted, however palpation of the stapes head did not produce a round window reflex Examination of the stapes footplate disclosed the absence of a complete annular ligament and removal of the mucosa around the anterior portion of the footplate showed that it blended into the bone of the otic capsule Elsewhere there appeared to be an indistinct separation of the footplate and otic capsule This footplate did not appear to be as thick as the footplate in case II. Small puncture holes were made around the stapes footplate and pressure upon the head of the stapes appeared to produce a round window reflex. No evidence of an aberrant facial nerve was noted and the fallopian canal appeared to be intact

Postoperative results

A one month postoperative audiogram revealed an average dB gain of approximately 15 dB

Comment

Audiometric examination preoperatively of both ears shows the effect of mass increase due to the presence of fluid behind the tympanic membrane. In the left ear it is superimposed upon a fixed stapes footplate This case illustrates a relatively thin congenital stapes footplate which was mobilized with considerable difficulty If refixation should occur a stapedectomy will be performed at a later date

Table 1 *Long term results of surgery on patients with congenital footplate fixation*

Case	Ear	Age At Operation	Pre-operative Loss Average Decibels	Surgical Procedure	Post-operative Average Decibels	Time
1	R	52	55	Mobilization (footplate shattered)	28 (ISO)	8 yrs
	L	50	57	Mobilization (footplate shattered)	52 (ISO)	9 yrs
2	R	32	58	Stapes Mobilization	20	2 1/2 yrs
	L		35	Stapes Mobilization	10	1 1/2 yrs
3	R	49	33	Stapes Mobilization	8	4 1/2 yrs
	L	50	50	Stapes Mobilization	12	5 yrs
4	R	10-1/2	40	Stapes Mobilization	20 (ISO)	8 yr
	L	24	43 (ISO)	Stapedectomy	0	3-1/2 mos
5	R	19	40	Mobilization	5	6 mos
	L	20	30	Mobilization	0	3 mos
6	R	17	40	Mobilization	5	15 mos
7	R	28	52	Mobilization	42 (ISO)	7 yrs
		35	63 (ISO)	Stapedectomy	38	1 yr
	L	36	60	Stapedectomy	17	1 yr
8	R	17	37	Stapes Mobilization (footplate shattered)	23	6 yrs
	L	14	13	Stapes Mobilization	55 (ISO)	9 yrs
9	R	47	40	Stapes Exploration	40	9 mos.
		48	40	Fenestration	30 (ISO)	6 yrs
	L	48	42	Mobilization	20 (ISO)	8 yrs
10	R	60	50	Mobilization	17 (ISO)	7 yrs
	L	62	78	Mobilization	43 (ISO)	3 yrs
11	R	32	37	Mobilization	10	2 1/2 yrs
	L	34	40	Mobilization	30	3 yrs
		35	30	Stapedectomy with Vel Plug	15	1 1/2 yrs
12	R	22	55	Mobilization	30	2 yrs
		25	30	Stapedectomy	80 (ISO)	3 yrs
	L	23	60	Mobilization	53	6 yrs
		24	63	Stapedectomy (drill-out with P E strut)	35	6 yrs
13	R	51	58	Mobilization	58	1 yr
		52	53	Stapedectomy	17	5 yrs
14	R	30	60	Stapes Exploration (mobilization attempted)	50	4 mos
		33	45	Stapedectomy with Wire Vein Plug	48 (ISO)	5-1/2 yrs
	L	31	48	Mobilization	19 (ISO)	4 yrs
15	R	49	50	Stapes Mobilization	22	1 yr
	L	54	30	Stapedectomy	18 (ISO)	2 yrs
16	R	46	50	Stapes Exploration	50	
		47	50	Fenestration	25 (ISO)	6 yrs
	L	48	48	Mobilization	8 (ISO)	5 yrs
17	R	60	73	Mobilization (shattered footplate)	50	5 yrs
			60	Stapedectomy	30	3 yrs
	L	6	55	Stapedectomy	57 (ISO)	2 yrs

LONG TERM SURGICAL RESULTS OF CONGENITAL FOOTPLATE FIXATION

This presentation includes the long term review and analysis of 30 ears operated on by Howard P House and represents the largest number of cases reported to date. The initial records of these cases have been made available through the courtesy of Howard P House Originally 26 cases of congenital footplate fixation were explored by tympanotomy These cases represented 1% of House s (10) mobilized cases.

The author has conducted his own research on the long terms results, and has presented his own analysis of these cases It will be noted that there is a difference in the number of the initial cases and the number of cases that could be reached for long term study This indicates the diffi culty encountered in any long term study in maintaining contact with patients. Contacting 17 patients out of 26 may be considered a better than average response

All of the 25 ears on which mobilization of the footplate was performed showed evidence of an initial hearing gain except two Of these 23 mobilized footplates, six cases showed evidence of refixation. Of the total 30 ears which had been operated upon either by stapes mobilization, stapedectomy or fenestration, 23 have maintained a practical 30 dB level or better at the most recent test.

Nineteen of the operated ears have maintained results for one year or longer and 12 have maintained the hearing gain for 3 years or longer The longest duration that hearing has been maintained in any one ear is 8 years.

COMMENT

All of these patients had good preoperative cochlear function indicating this to be a pure conductive loss. Although insufficient histologic studies have been done on the organ of Corti in cases of congenital footplate fixation, it is apparent that the cochlea was not involved in these cases. In contrast, otosclerosis may exhibit cochlear damage which is now thought to be due to vascular causes. Ruedi (44) has stated that the sensorineural changes are the result of vascular shunts which result in congestion of the cochlear veins.

Although this series of congenital footplates, which have been mobilized is very small as compared to the otosclerotic footplates which have been mobilized, there is less possibility of refixation in congenital footplates.

It would appear that the reason for this would be the absence of osteogenic activity such as would occur in an active otosclerotic focus.

In the ears in which stapedectomy was performed, there was no evidence of bony closure. This again would indicate a lack of osteogenic activity in the oval window area following surgery

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LONG TERM SURGICAL RESULTS OF CONGENITAL FOOTPLATE FIXATION

This presentation includes the long term review and analysis of 30 ears operated on by Howard P House and represents the largest number of cases reported to date The initial records of these cases have been made available through the courtesy of Howard P House Originally 26 cases of congenital footplate fixation were explored by tympanotomy These cases represented 1% of House s (10) mobilized cases.

The author has conducted his own research on the long terms results, and has presented his own analysis of these cases. It will be noted that there is a difference in the number of the initial cases and the number of cases that could be reached for long term study This indicates the difficulty encountered in any long term study in maintaining contact with patients. Contacting 17 patients out of 26 may be considered a better than average response

All of the 25 ears on which mobilization of the footplate was performed showed evidence of an initial hearing gain except two Of these 23 mobilized footplates, six cases showed evidence of refixation Of the total 30 ears which had been operated upon either by stapes mobilization, stapedectomy or fenestration, 23 have maintained a practical 30 dB level or better at the most recent test.

Nineteen of the operated ears have maintained results for one year or longer and 12 have maintained the hearing gain for 3 years or longer The longest duration that hearing has been maintained in any one ear is 8 years.

COMMENT

All of these patients had good preoperative cochlear function indicating this to be a pure conductive loss. Although insufficient histologic studies have been done on the organ of Corti in cases of congenital footplate fixation, it is apparent that the cochlea was not involved in these cases. In contrast, otosclerosis may exhibit cochlear damage which is now thought to be due to vascular causes. Ruedi (44) has stated that the sensorineural changes are the result of vascular shunts which result in congestion of the cochlear veins.

Although this series of congenital footplates, which have been mobilized is very small as compared to the otosclerotic footplates which have been mobilized, there is less possibility of refixation in congenital footplates.

It would appear that the reason for this would be the absence of osteogenic activity such as would occur in an active otosclerotic focus.

In the ears in which stapedectomy was performed, there was no evidence of bony closure This again would indicate a lack of osteogenic activity in the oval window area following surgery

CONCLUSION

1 Embryological and histologic studies have shown that congenital foot plate fixation is an entity resulting from the failure of the annular ligament to develop This is due to the retardation or failure of the normal maturation process in which the lamina stapedialis separates from the otic capsule.

2 Although studies have shown that in a greater number of cases of congenital deafness, only the middle ear or the labyrinth may be involved Some cases of congenital fixation of the stapes footplate with mixed deafness may occur such as in rubella

3 Diagnosis of congenital footplate fixation may be confirmed by audiometric findings, lack of progression and classical findings upon performing an exploratory tympanotomy

4 When possible the treatment of congenital fixation of the stapes foot plate should be mobilization of the stapes and if this is not possible, a stapedectomy may be performed.

5 Footplate fixation with complete fusion of the stapes footplate with the surrounding otic capsule should be treated by fenestration In children fenestration should be delayed at least until adolescence

6 This long term analysis of surgical results on congenital fixation of the stapes footplate has revealed that refixation or bony closure of the oval window is less likely to occur than in the cases of fixation of the stapes footplate involved with otosclerosis.

7 A wide search by the author among leading otologists and temporal bone laboratories in this country has failed to produce any histopathologic material The two places where histopathologic material was present were the Histopathological Laboratory of the Department of Otolaryngology (30) at the University of Chicago, and the temporal bone bank of the Los Angeles Foundation of Otology

8 More cases of congenital fixation of the stapes footplate may exist than we think, due to the fact that cases may exist in children which may not be diagnosed due to its relative rarity and possibly cases go unrecognized at the time of surgery due to the surgeon's unfamiliarity with this condition

9 Studies of the effect of various chemicals or virus agents on the genes, with subsequent inhibition of RNA synthesis and its effect upon the cell or organ at a particular stage of development may possibly yield the answer to the formation of a congenital fixation of the stapes footplate

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TYMPANIC MEMBRANE GRAFTS OF FULL THICKNESS SKIN, FASCIA AND CARTILAGE WITH ITS PERICHONDRIUM

An Experimental and Clinical Investigation

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TYMPANIC MEMBRANE GRAFTS OF FULL THICKNESS SKIN, FASCIA AND CARTILAGE WITH ITS PERICHONDRIUM

AN EXPERIMENTAL AND CLINICAL INVESTIGATION

BY

BENGT SALÉN

BODEN 1968 UMEÅ

To My Wife

Contents

I Introduction

During the last decade great efforts have been made to improve the tympanoplastic surgery introduced by Zöllner and Wullstein in the early 1950s To this end a large number of tissue materials have been proposed as the most suitable for drum grafts (Table 1) As a result of development in recent years there has been an increasing tendency to discard epithelium-covered grafts in favour of various tissues of a purely mesenchymal nature. That any one of these is definitely superior to the others has not been proved

During this development divergent opinions have been voiced for instance while Guilford (1962) declared the use of external body skin to belong to the historic past, Wullstein (1968) has defended the procedure pointing out that the occasionally unsatisfactory results obtained with this material were possibly due to deficient surgical technique rather than to shortcomings of the tissue That the views on the other tissues used in tympanic grafting are no less divergent is suggested by the fact that new types of graft have constantly been proposed, even when those already introduced by their advocates have yielded excellent results.

While several of these tissues have made only a transient appearance in the literature others have found comparatively frequent clinical use It seems, however reasonable to assume that no one otologist can personally perform enough cases to evaluate all these tissues from the aspect of tympanic closure and hearing restoration. Since only a few studies of the various types of tissues have been performed in laboratory animals, it has been necessary to rely on the clinical results reported by different otosurgeons. The fact, however that such reports have almost invariably been concerned with one particular graft tissue renders them unreliable as a basis for comparsion because the reported results will have been influenced to an unknown extent by various conditions, such as the state of the ears operated on the length of the follow up period, and the skill of the surgeon It is therefore understandable that there are still no truly representative comparisons between the varous types of tympanic grafts.

The present investigation was undertaken with the object of comparing autogenou full-thickness skin, fascia and elastic cartilage with its perichondrium with regard to their suitability as tympanic membrane grafts Of prime interest was the possibility of obtaining with these materials a permanent tympanic closure the result of the transplantation from the aspect of acoustic functi n was also assessed.

The tissue transplants were studied in experimental grafts in the cat and in connection with clinical myringoplastic operations in man. Answers were sought to the following questi ns

1 In what way and to what extent do these tissues integrate when transplanted to perforated but otherwise normal tympanic membranes?
2 In what way and to what extent do these tissues integrate when transplanted to post-inflammatorily fibrotic drum remnants?
3 If an integration and closure of the perforation has been obtained, what effect do these tissues have on the transmissions properties of the middle ear?

In an attempt to answer the first of these questions and perhaps to obtain enlightenment on the third the three tissues were used in experimental closure of fresh traumatically produced perforations of the ear drum in the cat. The membranes so formed were then examined from the aspect of morphology and function.[1] To answer the second question and possibly to gain a further insight into the third a uniform series of 232 clinical myringoplastic operations were followed up. In these operations the three types of tissue had been used consecutively and to about the same extent.

[1] These studies were performed in collaboration with Dr. Jan W[illegible]ll, Assistant Professor at the Department of Oto[illegible]logy, Karolinska Sjukhuset, Stockholm, and Dr. M[illegible] Bergstedt, Assistant Professor at the Department of Ot[illegible]logy, The University of Umeå, Umeå.

II Myringoplasty—Survey of the Literature

Historical notes

In 1878 Berthold reported 2 cases in which he had closed tympanic membrane perforations with free full-thickness skin grafts—with initial success. He designated this surgical procedure "myringoplastik" and stressed the importance of properly preparing the vascular bed. He also pointed to the difficulty of getting external body skin to survive in the warm moist environment of the bottom of the external auditory canal. Ely (1881) and Tangemann (1884) reported 9 and 2 similar operations, respectively, all of which were judged to be initially successful. After longer follow up periods, however, Berthold (1889) expressed negative views on the suitability of skin for myringoplasty and tried in a few cases to use instead corneal tissue from the rabbit.

Table 1. Drum graft tissues proposed since 1952.

Graft tissue	Author	Graft type
External body skin	Moritz (1952), Zöllner (1952), Wullstein (1952)	autologous
Amniotic membrane	Schrimpf (1954), Vertlib (1966)	homostatic
Meatal skin	Frenckner (1955), Plester et al. (1959)	autologous
Mucous membrane of the cheek	Hall (1956), Borisova (1965)	autologous
Dermis	Burian (1958)	homostatic
	Reed (1965)	autologous
Cornea	Holewinski (1958), Forman (1960)	homologous
	Flottes et al. (1963)	heterostatic
Periosteum	Claros-Domenech (1959), Becca et al. (1959)	autologous
Fascia	Örtegren (1959), Heermann (1961)	
	Jantsek (1966)	homostatic
Vein	Shea (1960), Tabb (1960)	autologous
	Schiff et al. (1963)	homologous
	King (1961), Williams et al. (1965)	homostatic
Peritoneum	Birch (1961)	autologous
Dura mater	Preobrazhensky (1961), Albrite (1966)	homologous
Adipose tissue	Ringenberg (1962), Sterkers (1964)	autologous
Perichondrium	Goodhill (1963)	
Cartilage with its perichondrium	Holmgren (1963), Jansen (1963), Salén (1963)	autologous
Pericardium	Trombetta (1963)	homologous
Tympanic membrane	Chalat (1961), Marquet (1966)	
Umbilical cord artery	Hsiao et al. (1961)	homostatic
Collagen	Salén et al. (1965), Patterson (1967)	heterostatic
Heart valve tissue	Cornish (1965)	homostatic

As the above attempts to close drum perforations with free tissue grafts apparently did not come up to expectations, interest in the method lapsed until the beginning of the 1950s, by which time situation had undergone a transformation. The use of external body skin was again advocated as a graft material in myringoplasty, this time by Zöllner (1952) and Wullstein (1952). In a few years, however, differences in opinion on its suitability for drum grafts led to the testing and clinical application of other tissues (Table 1).

Skin grafting

Wullstein (1952) started using free retroauricular skin grafts of three-quarters thickness, while Moritz (1952) and Zöllner (1952) preferred the pedicular type. Before long, however, Wullstein (1953) changed to the full thickness skin grafts and Zöllner (1954) abandoned the pedicle on realizing that it was of no value in the nutrition of the transplant. Guilford *et al* (1958) likewise advocated retroauricular skin but preferred the split-thickness type. House *et al* (1961) shared this opinion; they considered that with this material the conditions for incorporation should be better because *of the greater number of transected capillaries open to the vascular bed.*

Although the external body skin graft was the type in by far the most common use in myringoplasty throughout the 1950s remarkably few reports of results obtained with this technique were published, and of those many were deficient in various respects; for instance, only the functional or the anatomic results were reported, and the follow up period was often not given.

In general, the first results to be reported were good, but this situation did not last, as is evident in some measure from table 2. Lebo (1963) stated that in myringoplasty with full thickness skin postoperative perforations might be expected in 35 per cent, and still more if split thickness skin is used.

Wullstein (1959) maintained that full thickness skin cannot provide a functionally perfect eardrum. Because skin is acoustically inferior to the normal tympanic membrane, there will always remain an air bone gap of at least 15 dB. In an experimental study Allen *et al* (1960) found a moderate rise of the air-conduction threshold already at 500 Hz, at 4000 Hz the loss was about 20 dB.

When Peer (1955) pointed out that after a successful transplantation to a heterotopic environment skin will retain its biologic character unchanged this had long been a familiar fact. As external body skin differs both histologically and physiologically from the natural drum tissue, it follows that its use in myringoplasty may imply permanent implantation in the restored membrane of foreign structures such as glands and hairs.

In animal experiments Burian *et al* (1959) found that following a typical autotransplantation of external body skin the epithelial crests that are

Table 2 Reported percentage of closed perforations following external body skin grafting

Author	Year	No. of cases	Follow-up period (years)	Perforations closed (%)
FULL THICKNESS SKIN				
Bandtlow	1960	300*	3	91
Agazzi	1960	292	>1	79
Thorburn	1960	92	>0,5	70
Beales	1961	87	—	75
House et al.	1961	39	—	72
Guilford	1962	137	—	89
Mawson et al.	1962	62	1—5	41
Wright	1963	207	—	89
Černy et al.	1963	87	—	60
Mitchell	1967	27	>3	70
SPLIT THICKNESS SKIN				
House et al.	1961	31	—	72
Guilford	1962	195	—	68
Wright	1963	195	—	66
Örtegren	1961	20	2	15

*800 operated on, 300 followed up.

absent in the squamous epithelium of the eardrum not only penetrated deeper into the papillary portion of the dermis but also formed epithelial cysts there which closely resembled graft cholesteatoma.

Beickert (1958) and Schuknecht *et al* (1960) showed that glands and hair follicles can lead to perforations both directly and indirectly the former would be the case if these preformed ducts, lined with squamous epithelium, were transected on removal or tailoring of the graft, and the latter if they were widened so much through epithelitis or cyst formation that eruption into the middle ear resulted.—In the retroauricular skin, however Wullstein (1960) disputed the occurrence of such structures reaching into the deepest portion of the dermis.

The accumulation of desquamation products through the absence of any migratory power of the cells of external body skin is generally accepted as a factor predisposing for inflammatory reactions and the formation of cysts. Guilford (1962) found dermatitis in nearly 10 per cent and epidermoid cysts in about 3 per cent of the cases in whom full thickness skin had been used in myringoplasty and in a corresponding series Wright (1963) reported inflammatory changes and cysts in nearly 8 and 7 per cent, respectively

Thorough studies have been made of the development of the circulation in autogenous skin grafts. Conway *et al.* (1951) showed that during the first 24 hours the vessels of the graft are contracted, with no circulation, so that fluid exchange between graft and bed in this period occurs

only by diffusion according to Kley *et al* (1959) this plasmatic circulation is found as early as 20 minutes after myringoplasty. After about 24 hours, however the capillaries of the graft are dilated and some then lie in direct contact with the vascular orifices in the bed so that a "mouth to-mouth" circulation is established (Hynes, 1954). This is, however insufficient for the nutrition of the graft to ensure this it is necessary for vascular buds from the bed to penetrate into the graft but according to Converse *et al* (1950) there is a delay of about 3 days before blood begins to circulate in these new vessels. Then the corium is supplied at first, as is manifested according to Grünberg *et al* (1964) by the resumption of the metabolic activity of the connective tissue cells of the graft at about this time.

In ordinary external body skin transplantation Schäfer (1949) found regressive changes to be more common in full thickness than in split thickness grafts. The opposite situation that obtains in myringoplasty is due to the perforation as Smahel *et al* (1960) have shown experimentally. Because of the anatomy of the vessels in the skin in thin split skin grafts the part of the epidermis situated over the perforation will have no preformed vascular communications with the bed whereas in full thickness skin grafts these are provided by the vessels of the deep portion of the dermis.

The reduced vascular bed due to the presence of the perforation will not always meet the requirements of the skin graft, especially as regards the oxygen supply of the epithelial cells (Rambo 1961 Thorburn 1961 Culliford 1962 Goodhill *et al.*, 1964) degenerative reactions in the epidermal portion will ensue that decrease the resistance of the graft to bacteria.

Connective tissue grafting

The knowledge that the epithelium of the ear drum easily regenerates so long as the vascular lamina propria is intact, together with the experience gained in myringoplasty with external body skin led to a simultaneous, worldwide adoption of connective tissue transplants instead of epithelial grafts. The intention was to use the connective tissue to replace only the substance lost in the lamina propria leaving the corresponding defects in the surface epithelium to be covered by the regeneration from the environment. As a tissue graft suitable for myringoplasty should have a high survival capacity and, after integration bear a close resemblance to the aponeurotic lamina propria several types of connective tissues were tried out.

Periosteum from tibia has been recommended by Claros-Domenech (1959) and Bocca *et al* (1959) while Guerrier (1963) Berger *et al* (1965) and Wells (1965) preferred the same material but obtained from the mastoid region. Fascia was introduced by Ortegren (1959) Heermann (1961) and Storrs (1961) all of whom took the graft from the temporal muscle fascia.

Cartilage and perichondrium from the nasal septum has been used by Jansen (1963) and Salén (1963) while Goodhill (1963) advocated perichondrium from the tragus which Brockman (1965) also used.

While an external body skin graft can be adapted to the edges of the perforation in only one way the non-epithelial connective tissue graft can be attached to the outer or inner surface of the de-epithelialized drum remnants (Wright 1963) or placed between the connective tissue frame and the epithelium of the membrane fragments (Plester 1963)

From the literature it is evident that the results of myringoplasty with connective tissue were good from the standpoint of both function and closure of the perforation (Table 3) Austin (1964) obtained closure of the perforation in 95 per cent and Storrs (1966) in 90 per cent of their series. Using connective tissue grafts, Portmann (1965) also obtained functionally acceptable restauration in 82 per cent of his cases

From a histologic standpoint fascia, periosteum and perichondrium are extremely closely related variants of aponeurotic connective tissue being composed chiefly of fibrocytes and collagenous fibres deriving from them (Stearns, 1940)

The comparatively low oxygen requirement of connective tissue means that tympanic grafts of this material integrate more easily with a deficient vascular bed (Burian, 1958 Heermann 1961 Thorburn 1963) Moreover the relatively primitive nature of the connective tissues involving a certain degree of weakness in their biologic character facilitates the creation of a

Table 3 Reported percentage of closed perforations following connective tissue grafting

Author	Year	No. of cases	Follow-up period (months)	Perforations closed (%)
FASCIA				
Heermann	1962	55		90
Palva	1963	34	4—24	100
Harpman	1961	30		100
Örtegren	1964	87	24	81
Charland	1965	10		80
Nilsen	1965	27	6—30	78
Sa ary	1965	20		60
Storrs	1966	301	36	90
Wright	1967	97		98
PERIOSTEUM				
Wright	1963	20		100
Berger et al.	1963	200		98
Wells	1966	40		90
PERICHONDRIUM				
Goodhill et al	1964	15	1— 6	100
Brockman	1965	30	3—20	9[illegible]

restored drum resembling the natural tympanic membrane (Beickert 1962 Perret 1963 Portmann, 1964)

Although fascia, periosteum and perichondrium are almost identical histologically they differ slightly from the clinical standpoint Wullstein (1963) and Kley (1963) found that fascia grafts granulate too much thus retarding the regeneration of the meatal epithelium Because of a greater homogenity a graft of periosteum or perichondrium adapts itself better to the vascular bed than does the fascia transplant (Wright, 1966) This is true especially of perichondrium from the tragus, whose natural vaulting is directly adaptable to the outward concave surface of the tympanic membrane (Goodhill 1967) The prospective osteogenic tendency of periosteum was pointed out by Zöllner (1963) After identical observations Wright (1967) abandoned periosteum for fascia or perichondrium the latter is considered to display no chondrogenic tendency (Goodhill 1961 1967)

III Histologic Study of Experimental Tympanic Closure in Cats

by

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Introduction

Surgical closure of persistent tympanic membrane perforations by means of tissue transplantation is a generally accepted method, though the tissue originally recommended for this purpose, namely external body skin, is no longer in common use. Curiously enough, like the original skin, practically all the newer tissues that have been adopted as drum grafts have been tested directly on human subjects, without the preliminary studies on animals that are customary in other fields of reconstructive transplantation surgery. Even in the case of materials that have been in general clinical use for a period, parallel animal studies of their behaviour in closure of the ear drum perforations have been remarkably few. Withers *et al.* (1963, 1965) have examined autologous fascia, vein and canal skin in the cat, Cholat (1964) homologous tympanic membrane in the frog, Guilford *et al.* (1964) autologous vein in the dog, Richards *et al.* (1965) autologous and homologous vein in the cat, Williams *et al.* (1965) homostatic vein in the dog, Watson *et al.* (1965) autologous fascia in the cat, and Salén *et al.* (1965) and Patterson (1967) heterostatic collagen in the cat.

The object of the present study was to examine in laboratory animals the integration of some common types of autogenous tympanic membrane grafts, namely fascia, full thickness skin and cartilage with its perichondrium, when transplantated to perforated but otherwise normal drum remnants. The last two would appear not to have been used previously in experimental myringoplasty in animals.

Material and methods

The study was performed on 22 cats weighing between 2 and 4 kg. In 9 of these animals bilateral, and in 13 unilateral, a fresh perforation of the drum, produced traumatically, was closed with autogenous grafts of full-thickness skin, fascia and cartilage with its perichondrium.

In all 31 ears the operation and treatment were standardized. Anaesthesia was induced by means of an intraperitoneal injection of 42 mg pentobarbitone sodium (BP) per kg of body weight. Throughout the operation strict surgical sterility was maintained. The drum was reached from a retroauricular incision after the meatal canal had been opened about 4 mm laterally of the tympanic ring. A central perforation 3—5 mm in diameter was produced by excising part of the drum with Zöllner's tympanoplasty instruments; an operation microposcope (Zeiss Epitecknoskop) was used. The perforation was situated mainly in the more accessible ventral quadrants. After careful de-epithelialization of the outer surface of the drum remnant the defect was closed with the selected graft tissue. This was full-thickness skin in 10 ears, fascia in 10 and cartilage—perichondrium in 11.

The skin graft was a confetti sized piece from the canal wall at the place where it was transected about 4 mm outside the drum. In the cat the skin in this area is furnished with glands (Fig. 1) and hairs, and covered with stratified squamous epithelium whose border with the corium is rendered uneven by epithelial crests.

The fascia tissue was obtained from the outer fascia of the temporal muscle chiefly from the ventral portion where the aponeurotic character is most clearly evident.

The cartilage—perichondrium graft was taken from the elastic cartilage of the outer ear quite near the ear tip. From the approximately confetti sized piece of tissue one layer of perichondrium was removed. The retained layer was made to overlap the cartilage by about one millimetre; this had previously been tailored to the perforation.

The grafts were handled with the greatest care. The skin graft was placed over the fresh traumatic perforation with the corium approximated to the de-epithelialized outer surface of the drum remnant. A similar meatal apposition was obtained for the fascia graft. The cartilage—perichondrium graft was applied so that the cartilage lamina lay in the actual drum defect, with the bare surface medially and the side bearing the perichondrium facing externally, the overlapping perichondrium then adhering to the outer surface of the denuded drum remnant. No retaining pack was placed in the canal. The retroauricular tissues were sutured but not the canal wall. No antibiotics or chemotherapy were given.

After perfusion with formaldehyde (5 per cent aqueous solution) under general anaesthesia the cats were decapitated, 5 of those with a unilateral graft after a follow up period of 3 1/2 months and all the others after 6 months.

Preliminary postmortem evaluations of the results of healing were made immediately by means of the operating microscope. The temporal bone was then dissected, fixed in a 10 per cent solution of formaldehyde, decalcified and treated with celloidin. From each specimen about 300 microscopic sections were prepared, stained with haematoxylin and eosin or by Mallory's azan method and examined histologically.

Results

A preliminary examination at the end of the follow up period showed all 31 perforations to be closed In 30 ears the graft was identifiable

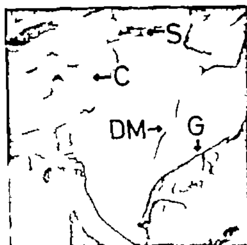


Fig. 1 Sectio through the middle ea f n treated rmal cat. DM, drum membrane S, stapes; C, cochl G gland in the canal w ll ski (Haemat xyli nd eo i) × 8



Fig. 2. The middl ear nd the healed drum membrane i cat 3 1/2 months ft ski graft. SG, ski graft. Cat ea no. 13a (M llory's aza) × 8

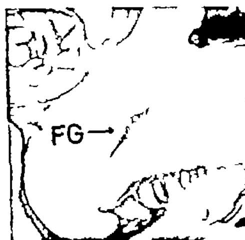


Fig. 3. The middl ea nd the healed drum membrane in cat 6 months fter f scia graft. FG, remainder of the fascia graft. Cat ar 753. (Haemat xylin nd eosi) × 8.

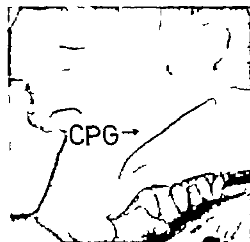


Fig. 4 The middle ea nd the healed drum membrane i cat 6 months after cartilage—perichondrium graft. CPG, cartilage—perichondrium graft. Cat ea no. 787 (M llory aza) × 8.

Full thickness skin

The macroscopic appearance of the skin grafts after closure is illustrated in figure 5. At the end of the follow-up period all 10 drums were healed, with no signs of infection. In 9 of them the grafts were seen in the ventral part of the transparent membrane as yellowish white, opaque and sharply demarcated areas. In one drum (nr 14s) no trace of the graft could be found. The approximation of the graft to the drum was good in 8 ears. In one (no 757s) it was marred by a small peripheral flap at the posterior

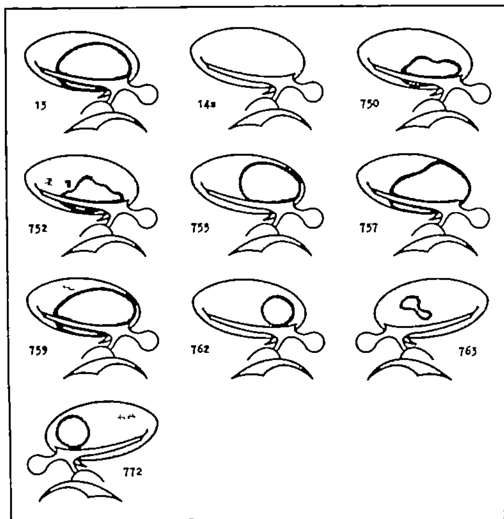


Fig. 5 Drum graft f f ll-thick ess skin l 10 cat ears.
F llow p pe od 3 1 2 months f nos. 13 nd 14 6 months f the others.
The drawi g bow the middl ears nd drums from the entromedi l part f the t mpa ic bulla, w th the imal supl The black tlined rea denot the configura t on f the i tegrated graft l cat ea no. 14 the graft is mi si g

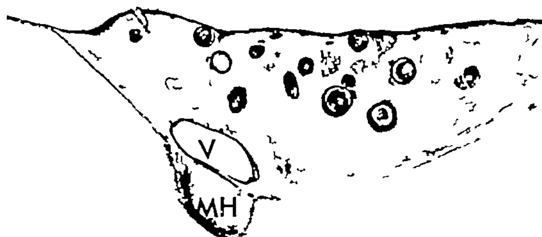


Fig. 6 Cat ear no. 13s.
Full-thickness skin graft in the drum membrane after 3 1/2 months. The squamous epithelium is penetrating the connective tissue with epithelial crests of varying size. Hair follicles and epithelial cysts are found deep in the dermal portion. A large vessel (V) is to be seen close to the handle of the malleus (MH). × 40

part of the graft lying just outside the drum membrane this flap was, however not inflamed and was normally epithelialized

In 9 ears histologic examination disclosed vital incorporation of the graft with the drum by reactionless union between the dermal portion of the graft and the lamina propria of the vascular bed In the ear (no 14s) in which there was no evidence of the graft the drum was quite normal in appearance The microscopic picture of the 9 incorporated skin grafts was fairly uniform (Figs 2, 6—8) Apart from a covering of the tympanal surface of the graft with an epithelium typical of the site all grafts had integrated without any considerable structural modification On the outer surface all showed thick keratinized squamous epithelium The subepithelial connective tissue contained both sebaceous glands and hair follicles (Figs 6 and 7) Hairs were sometimes seen to protrude from the surface (Fig 8) The border of the squamous epithelium with the underlying tissue was somewhat diffuse and irregular because of epithelial crests penetrating more or less deeply into the connective tissue Epithelial cysts filled with a horny mass were found below the surface epithelium Although these cysts were sometimes located deep in the graft there was no sign that they had erupted into

Fig. 7 Cat ea no 13a.
Higher magnificati f part f the ski graft seen i figure 6. The cyst i the g re filled with lamellated keratinous substance. On f the cyst i breaking th the meatal urface epithelium A n mber f tra sected hai f lli les re f ...
× 140



I g 8 Cat ea no 757
P rt f f ll-thickness kin graft ft 4 months. This i

the middle ear Some of them, however opened on the meatal surface of the graft (Fig. 7) In none of the grafts were inflammatory cells observed and the external auditory canal the middle ear and the inner ear were almost invariably free of inflammation

Fascia

The gross appearance of the fascia grafts is illustrated in figure 9 At the end of the follow up period all 10 perforations were closed and displayed no signs of infection or inflammation At the site of transplantation the other wise transparent drums were more or less opaque In one ear (no 764) the

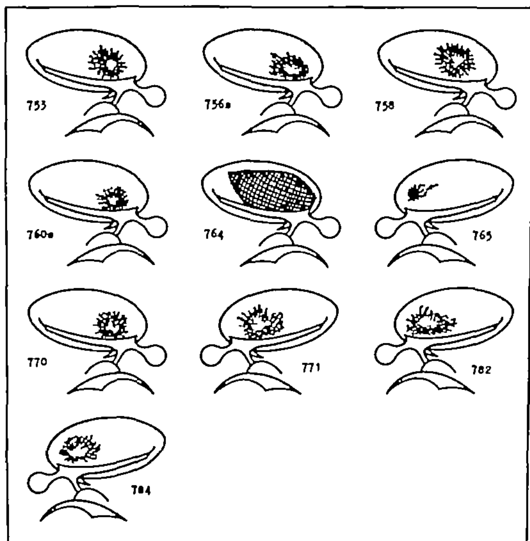
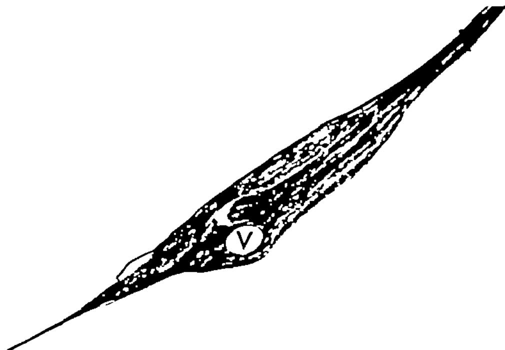


Fig 9 Drum graft f f sci l 10 cat ears. F ll w-up period 6 m ths. I t rpretati f the drawi g l figure 3



F g. 10 Cat ca no. 752.
The centre f the drum membrane 6 mo ths ft f scia graft. The histologic section how thi keni g f the subepithelial connective tissue. A regula poneurotic layering f the fibres i only partly isible A larg essel (V) i evidence f revascularization × 40

grossly unchanged graft had the appearance of a thick, nacreous, sharply demarcated area in the others, however there were only irregular fibrotic plaques, concentrated where the centre of the original perforation had been located, and becoming increasingly diffuse towards the periphery Thus, the border with the surrounding normal membrane was poorly defined in these specimens *In the sclerotic part of these drums there was a large winding vessel* (Fig. 10)

Histologic examination showed all the drums to be free of inflammation, and normally epithelialized on both outer and inner sides (Figs 3, 10 and 11) The graft (no 704) displaying a gross resemblance to fascia was found also under the microscope to have a lamellar structure typical of aponeurotic connective tissue (Fig. 11) To judge from the staining of the nuclei, the fibrocytes in thi connective tissue were vital In the other 9 membranes, however there was no such fascia like tissue but instead a diffuse and fairly small interspersion of a non-specific well organized and revascularized connective tissue This contained no inflammatory cells or foreign-body giant cells. The auditory canals and the middle and inner ears were all normal



Fig. 11 Cat ear no. 764
Part of the integrated grossl hanged f sci graft fter 4 mo ths. Th lamell rrangement of the f sci fibres nd the thin epithelium li i g both ides f the graft re seen x 250

Cartilage—perichondrium

The gross appearance of the cartilage—perichondrium grafts is illustrated in figure 12 None of the 11 drums in which this tissue was used displayed any macroscopic signs of inflammation at the end of the follow up period In each case the graft had the appearance of a sharply demarcated, opaque bluish-white lamina in the otherwise transparent membrane Approximation to the drum was excellent in 4 of the grafts (nos. 11s, 12s, 751 and 761s) acceptal le in 4 others (nos. 1os, 754s, 767 and 768) and rather poor in 3 (nos. 766 769 and 783) In the intermediate group a small peripheral part of the graft lay a little outside or in ide (no 1os) the membrane In the poorest group nly the ventral half of the cartilage was located within the membrane while the dorsal part had sunken into the middle ear where it was attached to the prom ntory wall by adhesions

Hist logic examination sh wed the drum grafts to be normally epithelia lized After organizati n the perichondrial component had integrated with the lamina pr pria in the same way a the fascia The cartilage comp nent on the other hand, wa n the wh le unchanged and situated in a connective tissue envel pe This consisted on the meatal side of a layer probably deriving fr m the perich ndrium and n the tympanic side of a thinner

layer of connective tissue regenerated from the remainder of the lamina propria. In 10 grafts the chondrocytes were vital (Figs 4 13 and 14) and in 2 of them (nos. 767 and 783) new cartilage had formed in places (Fig 16) In one graft (no 11a) the cartilage component consisted only of intercellular substance the chondrocytes being non vital The medial surface of this graft displayed fairly loose granulation with no giant cells. In the 3 ears (nos. 766, 769 and 783) in which most of the cartilage had sunken into the tympanic cavity (Figs. 15 16 and 17) and adhesions had formed between the graft and the promontory wall, these were in 2 ears extremely loose in structure and poor in cells (Fig 16) but in one ear (no. 766) fairly solid and

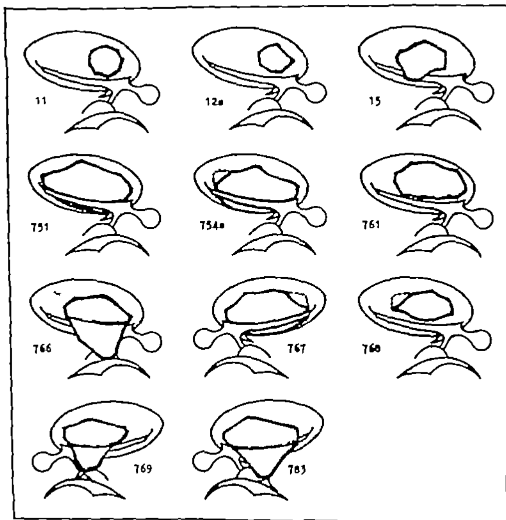


Fig. 12. Drum graft of cartil g with it peri hondri m l 11 cat ears. F llow-up period 3 1 2 months for nos. 11a, 12 nd 15 ; 6 months for th others. I terpret tion f drawi g l f gure 8 Not that I nos 766, 769 nd 783 the d rsal part f the tra spla ted cartilage lamina ha su ken I t the middl ea ca it wl re it ha become adherent to the promontory w ll.

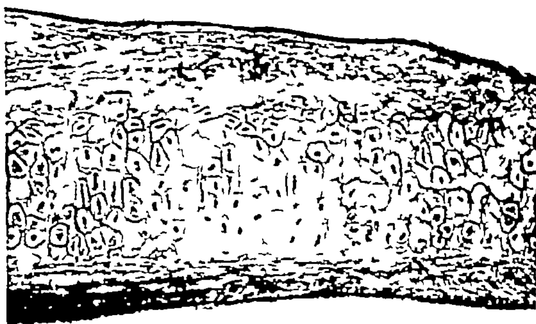


Fig. 13 Cat ea no. 767
P rt f incorporated cartilage graft ft r 8 m ths. A f can be j dged from the hi t logic picture the chondrocytes re ital O both ide the graft i covered with co nectl ti ue which in t rn i co red with pithell m × 330

Fig 14 Cat no 783
A sectio through th entral porti f partly di loc ted cartil g graft ft 6 months. Thi p rt f the graft w ll incorporated with the drum membrane nd appe rs t be complet ly it l
Cf figures 12, 1 d 16



Fig 15. Cat ea no. 783
A secti n through the dorsal part of the cartilag graft seen i figure 14 Thi portion f the transplant i partly sunken i t the middle ea cavity In spit f thi no perforation i t be seen. The entral rest f the graft is w ll incorporated with the drum membrane × 12.
Cf figures 12, 14 and 16.

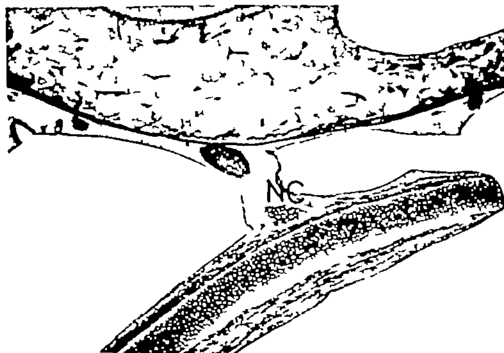


Fig. 16. Cat ea no. 783.
A section through the extreme dorsal segme t f the cartilage graft seen in figures 14 nd 15. This part of the graft is loosely connected t the promont ry w ll with mucosal fold the tra splant ppears t be ital nd newly f rmed cartilage (NC) is found the sid faci g the promontory × 40
Cf figures 12, 14 nd 15

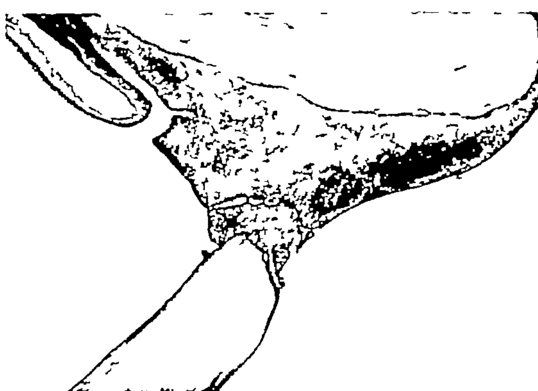


Fig. 17 Cat r 704.
A section thro gh the d rsal part f cartilag graft fte 6 mo ths. Thi segment f the graft i protrudi g freely i t the t mpa i ca it The gra l ti n ti e co ect i g the graft with the promont ry w ll co tain a larg umbe f lymphocytes, uggesti e f mild i flamm t ry reaction × 40
Cf figure 12.

containing accumulations of inflammatory cells (Fig 17) In this ear moreover there were granulations in the bulla tympanica Apart from this isolated middle-ear reaction there was in the other 10 cases no sign of inflammation in either the middle or inner ear

Discussion

It i often difficult if not impos ible to elucidate obscure problems in human medicine by means f experiments on animals, but in the present study which is concerned only with the behaviour of certain simple tissues in tympanic membrane autografts, the biologic background constituted by cellular tructures and the mode of reaction of the tissues is probably so nearly the same for most mammals that it is ju tified to a sume certain parallel between the c nditi ns in the cat and man

The advantages f animal experiments in the present context are obvious. The inc rp rati n r integrati n of vari us tis ue transplants can be com*pared under fairly tandardized c nditions the normally vascularized drum* remnant aff rd m st fav urable c nditi ns f r tympanic graft urvival

and after the animal has been sacrificed, the result of each transplantation can be examined most carefully under the microscope

The choice of laboratory animal was dictated by the need to work with a drum as large as possible compatible with ready availability and reasonable cost In these respects the cat is ideal, the area of the membrane being on average 42 mm² (Kirikae 1960)

The surgical procedure in the animal experiments followed the usual principles for myringoplasty in man Full-thickness skin fascia and cartilage—perichondrium were chosen for the grafts because these tissues have been commonly used in clinical myringoplasties. From the aspect of myringoplasty the meatal skin in the cat was considered to be comparable with external body skin in man because of their similarity in histologic structure

The follow up period—on average just less than 6 months—was considered long enough to provide an impression of the completed integration of the respective grafts. The same interval is often used clinically before evaluation of the results of myringoplasty Furthermore, preliminary experiments in the cat showed that subtotal traumatic perforations close spontaneously after only 2 or 3 weeks.

Full-thickness skin

The skin grafts had integrated with the surrounding drum with retained viability and character Sebaceous glands and hair were therefore incorporated.

Of greater interest, however is the fact that the squamous epithelium of the graft displayed signs of proliferation The border between the epithelium and the underlying connective tissue was therefore less regular after the transplantation than before A large number of epithelial cysts containing horny masses were developed within the dermal portion, possibly as a result of degenerative changes. Although these cysts were sometimes large and even opened on the meatal side of the graft there was no perforation found to the middle ear cavity

According to Beickert (1958) and Schuknecht *et al* (1960) among others, the cystic formations and the late perforations found after clinical myringoplasty are due mainly to the glands and hair follicles accompanying the graft. It is likely that even the majority of the cysts in the skin grafts of the present series originated from hair follicles and glands, but possibly also from more deeply penetrating crests of the proliferating squamous epithelium

As mentioned in Chapter II inflammatory processes in tympanic grafts of external body skin tend to increase the likelihood of secondary breakdown of the graft. A possible reason for the absence of perforations in the present series is then that the grafts did not display dermatitic reactions This is perhaps due to the fact that the skin grafts were taken from the auditory canal and that they were adapted to fresh, traumatically produced perfora

tions the grafted skin was therefore naturally suited to the environment of the canal—as regards both climatic conditions and possibly also the property of cell migration moreover the completely normal tissue of the drum remnants provided an ideal vascular bed from the aspect of myringoplasty

The absence of perforations and inflammation in the series may well have been temporary and due to the comparatively short follow up time the epithelial cords and the cysts of the squamous epithelium constituted loci minores resistentiae and therefore reperforation would probably have occurred if the follow up period had been extended

Fascia

When fascia was used only one graft integrated faultlessly that is to say with retained viability structure and volume At the site of the other 9 grafts there was only sparse atypical connective tissue

The changes that these 9 fascia grafts underwent are somewhat obscure According to Weis (1929) the fibre content and the arrangement of the fibres in the connective tissue are due to the mechanical stress to which the tissue is normally exposed It is then conceivable that there will be a decrease in the collagen content of a piece of fascia transplanted to an environment where it is subjected to less tension In the present study however this explanation would apply to all the other fascia grafts as well as these 9 According to Peer (1959) on the other hand autogenous fascia grafts retain their structure and character provided that the fibrocytes survive the transfer If they do not the succumbing graft will be replaced by connective tissue from the bed so that a tissue pattern identical with that of the host site results If this view is correct it is doubtful wether the fibrocytes of the 9 altered fascia grafts really survived the transplantation

How the superflous collagen is disposed by the organism is still not fully understood It has been shown by Lullinger *et al* (1942) in another connection that purely acellular collagen produces a foreign body reaction, and they inferred that the cause of certain chronic granulative processes in man was such a reaction to the collagen in a necrobiotic or otherwise altered connective tissue On the other hand, Peer (1955) states that he had never seen such a reaction in connection with autografts of fascia lata, nor has Salen *et al* (1965) using heterostatic acellular collagen in myringoplasty in animal experiments Curiously enough in 2 experimental studies on myringoplasty with autologous fascia Withers *et al* (1963 1965) does report finding a foreign body reaction, with giant cells in the graft zone

In the present series no foreign-body giant cells were found, and it would appear that superfluous collagen is normally accepted and absorbed

Cartilage—perichondrium

When elastic cartilage with adherent perichondrium was used the perichondrium integrated in the same way as the fascia, whereas the cartilage was enveloped by connective tissue and without any remarkable structural modification.

The feasibility of cartilage grafting has long been questioned. Loeb (1926) was the first to consider it established that autogenous cartilage with adherent perichondrium could in fact be transferred from one site to another with retained viability a view that was shared by Peer (1939) Young (1941) showed that even small pieces of cartilage from which the perichondrium had been removed could survive transplantation, the connective tissue of the host site then being converted to perichondrium Billingham (1954) however expressed his doubts maintaining that most of the chondrocytes die at an early stage because the cartilage tissue has no vessel bound circulation The animal experiments by Davidson (1959) have convincingly shown, however that at least elastic cartilage with its perichondrium can survive and retain its normal tissue structure when transplanted as an autogenous graft in favourable transplantation sites. This is presumably due to the low oxygen needs of cartilage tissue which, according to Krebs *et al* (1948) are considerably lower than for other tissues.

In the present series the viability of the cartilage varied both within and between the grafts In the periphery there were occasionally degenerated and even non vital chondrocytes, though new formed, vital cartilage was also found. One graft was devoid of vital chondrocytes after only 15 weeks, but the other 10 were quite vital after twice as long.

That the cartilage was so often viable is surprising in view of the fact that in the animal experiments the cartilage grafts were placed so that a completely naked surface faced the air filled cavity of the middle ear The explanation may well be that immediately after the operation the cavity was filled with blood and tissue fluids, which served as a medium from which the chondrocytes could obtain nourishment by diffusion

The remarkable tendency for demarcation of vital autogenous cartilage grafts from the surrounding tissues Peer (1963) ascribes to the fact that the intercellular substance of the vital cartilage is so well accepted by the organism If however the chondrocytes die the matrix is no longer tolerated and it is not long before it is invaded and absorbed by host granulation tissue The reversal of the behaviour of the environment to the cartilage is attributed to the fact that when the chondrocytes die the matrix undergoes chemical modification

In all but one graft of this series the vital cartilage was accepted well with no igns of inflammation In the exceptional case however granulations with lymphatic cell accumulations were observed adjacent to the cartilage here *an infectious origin cannot be ruled* out, since granulations were also found in the tympanic bulla. In that case, on the other hand, where the cartilage

was non vital there was still no more than a local trace of connective tissue invasion

In more than one half of the cartilage grafts the approximation to the drum was not entirely satisfactory One possible reason for thi is that owing to the irregular curvature of the drum the perforation rim did not lay in one plane and the plain cartilage lamina then could not easily be adapted to the rim along the whole of its perimeter Another possible reason, suggested by the sections, is that when the adherent perichondrium was organized the scar tissue shrinkage caused the cartilage to bend with the convexity inwards

Conclusion

The results of the study sugge t the following conclusions as regards the behaviour of the three graft tissues when experimentally transplanted to sound comparatively well vascularized drum remnants in the cat

(a) Free autogenous grafts of *full thickness skin* usually integrate with retained viability size and character After transplantation the squa mous epithelium of the graft tends to proliferate and as a result of this the grafts are penetrated not only by the ordinary hair and gland struc tures but also by newly formed epithelial strands and cysts.

(b) Free autogenous grafts of *fascia* integrate only rarely with a retained size and structure Instead most of them undergo such an extensive modification of character and form that the survival of the fibrocytes of fascia grafts is open to doubt

(c) Free autogenous grafts of *cartilage with its perichondrium* usually inte grate—the cartilage in an unchanged and for the most part viable form but the perichondrium like fascia, in a reduced state The incorporated cartilage is sometimes not perfectly adapted to the restored drum

IV Cochlear Microphonic Response after Myringoplasty

An experimental study on the cat

by

Martin Bergstedt M D and Bengt Salén M D

Introduction

In the space of the last decade more than a dozen tissues have been proposed for drum grafts. Their suitability has been indicated in the reports of the results achieved with them in clinical series There have however been few basic comparative studies of the various graft tissues in laboratory animals those that have been published have been concerned largely with how a particular tissue integrates with the graft bed, while the acoustic properties of the membranes healed in this way has been completely neglected

The experimental study reported here was undertaken with the object of ascertaining whether drums, that have been reconstructed with autogenous grafts of full-thickness skin, fascia or elastic cartilage with its perichondrium function differently as regards transtympanal sound transmission.

To obtain a comparable measure of the ability of the middle ear to transmit air-borne sound to the inner ear no knowledge of the auditory threshold was considered necessary Instead, the transmission properties of the middle ear were assessed on the basis of the relations that Wever *et al* (1936) found to exist between sound intensity and the electrical response of the cochlea—a method that in studies of the transmission properties of the middle ear has been used by among others, Bordley *et al* (1937) Wever *et al.* (1948) Wever (1950 1959) Lawrence (1950 1960) Payne *et al* (1951) Simmons *et al* (1962) Møller (1963, 1965) and Allen *et al.*, (1964)

Material

The normal r reference material for the study was composed of one ear of each of 10 young adult cats not previously submitted to operation and with no signs of wax, squamous products or local inflammation in the external auditory canal

Th experimental series consisted of 17 adult cats in sound health, in which myringoplasty had been performed 6 months previously — in one ear

in 8 of them and in both ears in 9 Full thickness skin was used in 8 ears, fascia in 10 and elastic cartilage with its perichondrium in 8 ears The histologic picture of the grafts have been reported elsewhere (Chapter III and Salén *et al* 1966)

The experimental material was grouped with respect to the type of graft and in each such group with respect to the size of the integrated transplant and any postoperative changes in the middle ear

Methods

Apparatus

The sound generating system consisted of a beat frequency oscillator (Brüel & Kjaer 1022) and an Elega 8-ohm loudspeaker The sound was carried to the animal via a 100 mm long rubber tube tightly fitted into the transected cartilaginous ear canal The rubber tube had an inner diameter of 3 mm and its 1 mm thick wall was conically tapered towards the meatal orifice of the tube within a 3 mm wide zone

The cochlear microphonics were picked up by a 100 μ platinum wire resting on the round window membrane Around parts of the platinum electrode a glass rod had been cast and this was attached to a stand by several articulated arms this arrangement prevented other than the required contact between the electrode and the animal The reference electrode was a stainless steel hypodermic needle located in the trapezius muscle The potentials were amplified 1000 times in a Tectronix 122 AC pre-amplifier and displayed on a Tectronix M 502 oscilloscope

During the experiment the cat was kept in a ventilated, sound insulated and electrically shielded box

The apparatus for stimulation of the cochlea was calibrated separately and after the experiments on the animals The sound-generating system was then connected to an artificial test ear resembling the cat ear with a coupler volume of 1 0 cm³ In order to determine the frequency response of the sound-generating system the sound pressure level near in the coupler was measured by a probe microphone (Brüel & Kjaer 4134) This was calibrated by a probe microphone coupler (Brüel & Kjaer UA 0010)

In the calibration the frequency relationship between sound pressure level and the electrical output of the beat frequency oscillator was that shown in figure 18 the measured sound pressure level was found always to be proportional to the voltage output of the oscillator

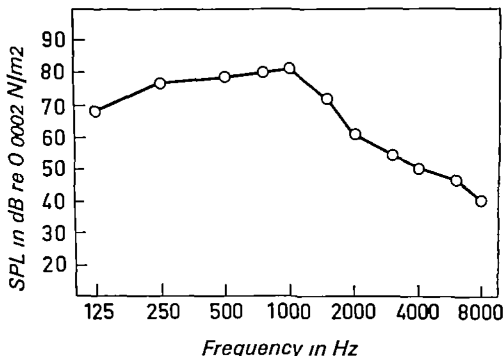


Fig 18. So nd pressure lev l as f ncti f frequency f 1.2 mV RMS lectrical utput f th beat frequency oscill t The mea urement, by calibrated probe microphone, wa perf rmed with the sound-ge rati g ystem co nected t rtificial ea

Procedure

Anaesthesia was induced with an intraperitoneal injection of pentobarbitone sodium (BP) The dose 42 mg per kg of body weight permitted satisfactory abdominal respiration Oxygen was added to the inspired air continuously throughout the experiment and hypoxaemia due to obstruction of the respi ratory tract was avoided by fixing the tongue in the fully protruded position

From a retroauricular incision the bulla tympanica was reached by a ventrolateral approach The parotid gland was removed, and the digastric muscle and hyoid chain were partly excised. The bulla was opened fairly wide so as to afford easy access to the round window The bony septum, however that in the cat almost completely screens the bulla from the middle ear cavity was left intact. The external auditory canal was transected 4 mm outside the tympanic ring at the level where the fixed cartilaginous canal lead towards the mobile part After careful haemostasis and microscopic examin tion of the tympanic membrane the rubber tube connected to the loudspeak r was inserted into the canal stump As its walls in the contact zone diminished in thickness towards the orifice the tube could be inserted int the transected canal deep enough to obtain a stable and tight connection Because f the variation in width of the auditory canals in the various ani mals, the end of the tube was situated 2—3 mm from the plane through the

tympanic ring. So as to eliminate any possibility of leakage the joint between the tube and the canal was sealed regularly with animal fat.

With the aid of a binocular operation microscope (Zeiss Epitecknoskop) the bare tip of the platinum electrode was brought into contact with the centre of the round window membrane. The bone defect in the wall of the bulla was left open. Just prior to the registrations, when all the instrument had been adjusted, the box was placed over the insulated operation table.

Pure tones were applied to the ear by the loudspeaker and the intensity of the sound was adjusted to produce a standard cochlear microphonic response of 3.5 μV RMS. The sound level producing this response was recorded. The pure tones used were 200, 500, 700, 1000, 1500, 2000, 4000, 6000 and 8000 Hz.

Report of examination results

Reference series — The sound intensities that gave a cochlear microphonic response of 3.5 μV RMS at the various frequencies at the round window of the 10 normal ears are reported indirectly as the voltage output of the beat frequency oscillator expressed in decibels; the attenuator was related to a zero level equivalent to an electrical output of 1.2 mV RMS. The results are presented in tables and as sensitivity curves.

Experimental series — The sound intensities for the test ears, expressed indirectly as the voltage output of the beat frequency oscillator which gave a cochlear microphonic response of 3.5 μV RMS at the round window, are reported relative to the corresponding means for the reference material. The differences between the values recorded for the experimental series and the comparative values in the reference series are given in decibels re 1.2 mV RMS, and designated a threshold shift or a change in sensitivity. A plus sign denotes that the stimulation necessary to produce the standard cochlear microphonic response was higher than the mean for the reference material and a minus sign the reverse. The results for the 3 test groups are reported separately and they are presented in tables and graphically.

Results

Reference series

The sound intensities that gave a cochlear microphonic response of 3.5 μV RMS in the 10 ears not previously operated upon are reported for each ear and frequency in table 4. The table also gives the mean of the individually measured stimulation intensities calculated for each frequency. The mean sensitivity curve based on these means is given in figure 10, where the range is also indicated. For the respective frequencies in the order 200—8000 Hz the ranges were 18, 13, 17, 11, 11, 15, 17, 12 and 25 dB; on average for the 9 frequencies it was 15 dB.

Table 4 The beat frequency osc llator output required to produce cochlear microphonic response of 3.4 μ \ RMS dB re 1 2 m\ RMS Round window recordings. T nireated ears in 10 healthy cats.

Ea	Frequency in Hz								
\	200	500	700	1 000	1 500	2 000	4 000	6 000	8 000
718	—10	—17	—21	—21	—25	—16	— 5	— 3	0
727	— 3	—13	—14	—24	—23	—20	—15	—13	—10
731 s	— 3	—18	—21	—26	—25	—20	—12	—10	0
734	— 6	—20	—21	—25	—24	—23	—14	—13	+15
754	—13	—25	—31	—35	—31	—25	—13	—12	— 8
755	—15	—18	—27	—29	—28	—23	—21	—15	— 2
758	— 8	—18	—20	—32	—25	—10	— 5	— 3	— 2
759	0	—12	—20	—25	—20	—18	— 4	— 7	+10
760	— 5	—25	—27	—32	—27	—18	—13	—13	—10
61	—18	—24	—28	—32	—26	—19	—11	—12	— 2
Mean alue	— 8	—19	—24	—28	—25	—19	—12	—10	— 1

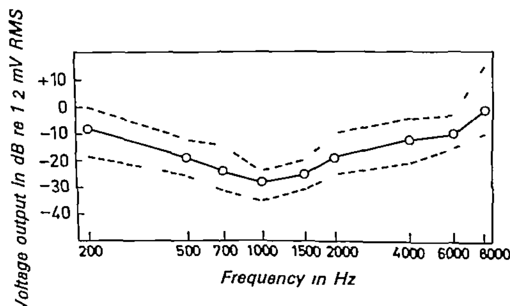


Fig 19 Se sit ty curves f 10 ears in normal cats.
O———O Med l es I t rquartil ra g

The se sit t or threshold i defi ed the ltag tp t (dB re 1.2 m\ RMS) f the beat frequ cy oscill tor required t produce cochlea microphonic response of 3.5 \ RMS, recorded t the round window

Experimental series

Full-thickness skin

For the 8 ears on which myringoplasty with full thickness skin had been performed the differences in the required intensity of cochlear stimulation, relative to the corresponding mean for the reference material are given in table 5 From results obtained for the ears in this group the mean sensitivity

Tabl 5 F ll-thickness ski ; 8 ear ears fter myringoplasty Threshold shifts (dB) relative to the means for the reference series

Ear No.	Frequency in Hz									Size of graft (mm²)
	200	500	700	1 000	1 500	2 000	4 000	6 000	8 000	
762	− 8	− 3	− 1	− 2	0	−10	+ 2	+ 4	− 1	
03	0	− 5	− 6	− 2	0	+ 7	+17	+ 8	+ 9	<10
772	+ 8	− 6	− 1	− 3	+ 6	0	+ 3	0	+ 1	
750	+ 8	− 1	0	− 6	+ 1	+ 7	+17	+ 8	+11	
752	+ 8	+ 1	0	0	+ 1	+11	+ 8	+15	+11	10−20
755	− 2	+ 3	0	− 1	0	− 3	+ 8	+11	+13	
757	+33	+25	+28	+27	+21	+29	+29	+27	+21	<20
759	+29	+21	+21	+28	+10	+39	+17	+55	+61	

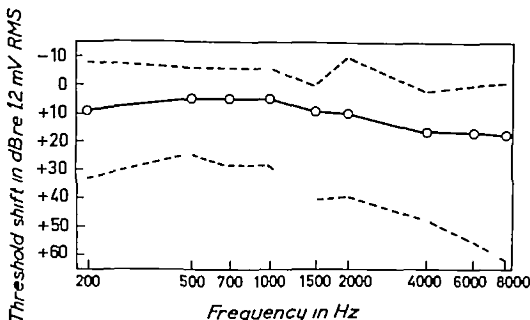


Fig. 20 Full-thickness ski graft ; 8 t st ears—Cha g i se siti ity
○———○ Medi l es I t rq rtil range

The se siti ity threshold i defi ed a the volt ge iput (dB re 1.2 mV RMS) f the beat freq ency oscillat req ired t prod ce cochlea microph i respo se f 3.5 µV RMS, recorded t the ro d wi dow

shift was calculated for each frequency these are illustrated in figure 20 From this figure it is seen that the voltage output of the beat frequency oscillator after myringoplasty with full thickness skin had to be increased by a mean of 10 dB for the 9 relevant frequencies in order to produce the standard cochlear microphonic response (3.5 µV RMS) The reduction in sensitivity was more pronounced at the higher than lower frequencies at 4000 Hz it was 16 dB, at 200 Hz 9 dB and at the frequencies of 500 1000 and 2000 Hz the mean was 7 dB

Fascia

For the 10 ears on which myringoplasty had been performed with fascia grafts, the differences in the required intensity of cochlear stimulation, relative to the corresponding means in the reference series, are given in table 6 From results obtained for the ears in this group the mean sensitivity shift was calculated for each frequency these are illustrated in figure 21 From this figure it is seen that the voltage output of the beat frequency oscillator after myringoplasty with fascia had to be increased by a mean of 4 dB for the 9 relevant frequencies in order to produce the standard cochlear microphonic response (3.5 µV RMS) The reduction in sensitivity was slightly greater at the higher than lower frequencies at the frequencies of 500 1000 and 2000 Hz the mean was 2 dB

Table 6. Fasci 10 cat ears after myringoplasty Threshold shifts (dB) relative to the means for the reference series.

Ear No.	Frequency in Hz									Size of grafts (mm²)
	200	600	700	1 000	1 600	2 000	4 000	6 000	8 000	
753	+ 3	+ 6	+ 2	— 2	— 3	+ 7	+ 3	+ 8	+ 6	
756	+ 3	— 1	+ 4	+ 8	+ 7	+ 1	+ 7	+ 3	+ 4	<10
758	+14	— 1	+ 4	+ 4	+ 5	+26	+27	+37	+32	
760	+ 8	— 1	— 2	— 1	+ 2	+ 9	+ 9	+10	+ 6	
761	+ 7	+ 9	+ 9	+ 6	+ 2	— 2	+ 6	+ 2	+ 6	>15
63	+10	+ 8	+ 3	+ 4	+ 6	+ 4	+ 5	+ 8	+ 9	
770	— 9	— 7	— 4	— 2	— 2	— 1	+ 4	0	+ 1	
771	— 3	— 9	— 6	— 2	— 1	— 2	+ 7	+ 6	0	<10
782	+13	— 1	+ 2	+ 1	— 2	+ 1	— 2	+ 2	+ 9	
781	+ 8	— 5	— 4	+ 1	+ 4	+ 6	+ 7	+ 9	+12	

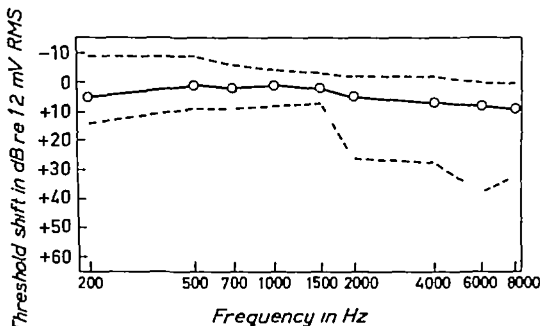


Fig. 21 Fascl graft 10 test rs—Cha ge i sen iti it
o———o Medi lues 1 t rquartile ra g

The sensitivity threshold i defined the v lt ge utput (dB re 1.2 mV RMS) f the beat frequency scill t r req ired t produce cochlea microphoni respo se f 3.5 μV RMS, recorded t the ro nd wi d w

Elastic cartilage—perichondrium

For the 8 ears on which 6 months earlier myringoplasty had been performed with elastic cartilage—perichondrium grafts the differences in the required cochlear stimulation relative to the corresponding means for the reference series, are given in table 7 From results obtained for the ears in this group the mean shift in sensitivity was calculated for each frequency these are illustrated in figure 22. From this figure it is seen that the voltage output of the beat frequency oscillator after myringoplasty with elastic cartilage—perichondrium had to be increased by a mean of just below 6 dB for the relevant 9 frequencies in order to produce the standard cochlear microphonic response (3.5 μV RMS) The reduction in sensitivity was greater at the higher and lower frequencies than at the intermediate ones at 4000 Hz it was 13 dB at 200 Hz 9 dB and at the frequencies of 500 1000 and 2000 Hz the mean was about 3 dB

Table 7 Elastic cartilage with its perichondrium 8 cat ears fter myringoplasty Threshold shifts (dB) relative to the means for the reference series.

Ear No	Frequency in Hz									Size of graft (mm)
	200	500	700	1 000	1 500	2 000	4 000	6 000	8 000	
51	+ 3	+ 3	+	+ 4	+10	+15	+16	+ 1	−2	
754	+ 6	+14	+12	− 4	+ 9	+11	+17	+13	+16	>20
761	− 1	− 1	− 4	− 2	+ 8	+ 3	− 5	− 3	0	
767	+23	+ 4	+ 4	0	+ 4	+ 4	+18	+12	+ 5	
768	+13	+ 9	+ 4	+ 6	+ 7	− 1	+ 7	+ 3	− 3	<20
766[1]	+13	− 1	− 1	− 4	0	+ 7	+13	+20	−16	
769[1]	+ 8	+ 9	+ 4	− 1	+ 1	+ 6	+22	+18	+ 9	>20
783[1]	+ 4	− 7	− 1	− 1	+ 1	+ 1	+ 9	− 8	− 6	

[1] Adhesions between the graft nd promontory wall

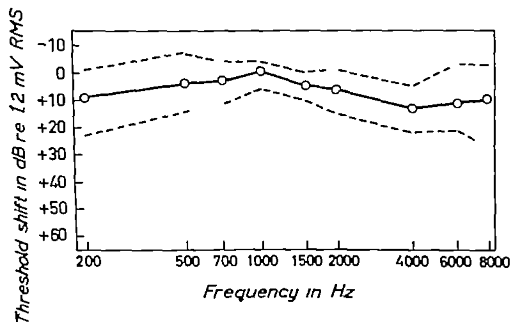


Fig. 22 Cartilage—perichondri m 8 test ears—Cha g in sensiti ity
O———O Median lues I terquartile range

The sensiti ity or threshold i defined the oltage output (dB re 1.2 mV RMS) f the best freque cy oscill t required t produce cochlea microphonic response of 3.5 µV RMS, recorded t the round w ndow

Discussion

The method of trying to solve clinical problems by means of animal experiments has both advantages and disadvantages. It is true that the problems can be clarified and the examinations made more efficiently in a manner that would be inconceivable in clinical studies, but as the results cannot often be applied directly to a human medical pattern the greatest caution is called for in drawing any conclusions.

While animal experiments have been a great aid in widening our knowledge of the normal function of the human ear in the case of the functional conditions in pathologically altered ears such experiments have often proved futile because most such conditions do not have their true counterparts in animals.

In the present study the object of which was to compare only a few different drum graft tissues often used in human medicine an experimental study on the cat was judged to be valuable because on this elementary plane there should be some agreement—as regards both morphology and function—between the conditions in the ears of the higher mammals.

The drum grafts that were tested functionally in the 20 cat ears were of full thickness skin fascia and elastic cartilage perichondrium. These types were chosen because they have been used fairly often from time to time in clinical myringoplasty and because they differ considerably in structure. As the intention in this investigation was to examine not the tissues in themselves but the results of the grafts in which they were used a period of 6 months was chosen for the interval between the transplantation and the functional tests. This period should be long enough to ensure that the healing processes were completed and that the reconstructed drum was structurally stationary.

For comparison of the transmission properties of the middle-ears where drum grafts had been performed with middle-ears not operated upon it is, of course, possible to determine and compare the auditory thresholds. In the case of the cat this means that the auditory acuity must be determined on the basis of conditioned reflexes—for instance—by the method reported by Neff (1947). Such a procedure is time-consuming and laborious, however and for the unpractised investigator not very reliable all animals cannot for instance be habituated and even when this is possible the intensity of the reflex-eliciting stimulus is still influenced by various conditional factors. For this reason it was decided to use the cochlear microphonic response instead of the hearing as the reference function. Within a certain range the magnitude of this function is proportional to the pressure of the stimulating sound (Wever *et al* 1949). In the cat the cochlear microphonic response is directly proportional to the volume displacement of the cochlear fluid (Møller 1963).

The apparatus used for this purpose functioned well and the reproducibility was satisfactory.

For the stimulation a closed system was used to lead the sound from the loudspeaker to the ear drum The advantages of this arrangement over the open tube method in which the end of the tube is situated 2.5 cm outside the ear are that distortion is less and that through removal of the external ear the drum region is more easily accessible for observation and cleaning (Wever *et al.*, 1954)

Since the magnitude of the cochlear microphonic response recorded from the round window varies quite widely with the position of the elec trode in the region (Møller 1963) in comparative studies it is necessary to ensure reproducible positioning of the electrode and for this reason the tip of the platinum wire used in these experiments was placed in the centre of the round window membrane

The cochlear microphonic response to which the sound stimulation was always referred was 3.5 μV RMS (10 μV p—p) This value is often used in similar studies and it was also suitable for the local conditions because this relatively low potential difference could always be distinguished clearly in the oscilloscope from the constant background noise

As regards the intensity of the sound that gave the standard cochlear microphonic response this was measured indirectly as the voltage output of the beat frequency oscillator for the purpose of the comparative study the absolute value of the sound pressure level was considered to be of only secondary importance

To assess the transmission properties of the ears in which myringoplasty had been performed all were grouped and each group correlated with the mean ear of the reference series. The correctness of this procedure is open to discussion The volume and configuration of the middle ear cavity the elasticity of the walls, and the diameters of the drum and canal vary from one animal to another As these factors, which were not determined here influence the individual measurement results, the bases for comparison could be fairly unreliable But the autocorrelative method, too, has its disadvantages, which in the present context are considerable exposure and opening of the bulla tympanica at the same time as the experimental myringoplastic operation might damage the arteries of the region, and infections or traumatic lesions can give exudative and granulative proc esses in the bullar part of the middle ear The former then perhaps have a detrimental effect on the circulation in the vascular bed possibly leading to impaired incorporation of the graft, while the latter perhaps gives rise to postoperative changes in the niche of the round window such that the cochlear microphonic response recorded after the transplantation is more or less difficult to relate to that before the myringoplastic operation As, finally the present study was not intended to provide an exact picture of the effect of the various drum grafts on the transmission properties of the middle ear but only to give an impression of these conditions, the mode of comparison used was judged to be applicable

As regards the results it may be mentioned that the sensitivity curves for the 10 normal ears composing the reference material could not be recorded with equal stimulation intensities. The difference between the strongest and weakest stimulus that gave rise to the standard cochlear microphonic response in these 10 ears—that is, the range—was, on average 15 dB for the 9 frequencies—a value that exceeds by only 2 dB the range reported graphically by Allen *et al.* (1964) for a similar series. As mentioned earlier this range is probably due to anatomic and structural differences between the ears in question. An important factor in this connection is the bony septum that almost completely separates the bulla from the middle-ear cavity in the cat. If the bulla is left open but this septum is persistent as was the case in the present series, the impedance will suddenly and transiently increase at the frequency of the principal resonance of the middle-ear cavity while there is a parallel decrease in transmission—a phenomenon that almost disappears if this septum is removed (Møller 1965). Although the principal resonance frequency of the middle-ear cavity in the cat is about 3 500 Hz there are fairly large individual variations owing to the normal anatomic variations, and this must be taken into account when results obtained in the different animals are compared. In the study quoted above (Allen *et al.*, 1964) this septum was not persistent and this partly explains the difference in results. Other factors that may have *contributed to the ranges of the sensitivity curves of the reference series* is the anaesthetic used and the fact that tracheotomy was not performed. The barbiturates affect the middle ear muscles and hence the transmission to a comparatively marked extent (Møller 1965). Any differences in the sensitivity to anaesthesia in the animals can therefore have given deviant recordings. As the cochlear potentials diminish with falling tissue oxygen tension obscure hypoxaemia in an animal can also have led up to random results.

When the ears with drum grafts were compared with the mean middle *ear in the "normal" reference series the following was observed*. The test group in which myringoplasty was performed with *full thickness skin* was characterized as a whole by a general reduction in sensitivity over the whole of the frequency range examined. This functional impairment was slightly more marked at the higher than the lower frequencies and it was least pronounced at frequencies around 1 000 Hz. From an examination of the individual cats in this experimental group it is seen (table 5) that the general reduction in sensitivity or transmission occurring in the mean ear was accounted for largely by the ears 757s and 759s. In both these the drum grafts were quite large but this factor alone can hardly account for the marked general reduction in sensitivity in these ears. If this point is disregarded, however it would seem that in both these ears, as in the other 6 of the experimental group there is evidence of the effect on the middle-ear transmission indicated above—namely slight impairment of the transmission at 200 Hz (about 3—9 dB) and to

a somewhat greater extent in the range 4 000—8 000 Hz (about 0—10 dB) whereas around 1 000 Hz the impairment was hardly anyone at all.

In the test groups where the graft tissue was *fascia* or *elastic cartilage with its perichondrium* the sensitivity curves appeared to follow largely the same pattern as was recorded for skin grafts though they were slightly flattened for fascia (fig 21) and accentuated for cartilage—perichondrium (fig 22) This means that the impairment of the transmission with fascia —which was moreover small—was more uniformly distributed over the frequency range while the impairment with cartilage—perichondrium was slightly greater in the base and treble regions, but slightly smaller at 1 000 Hz, where the transmission appeared to be practically normal This applied remarkably enough also to the 3 ears (nos 706 709 and 783) in which loose adhesions had formed postoperatively between the cartilage and promontory wall

The above impairments of the ability of the middle ears to transfer air conducted sound are presumably due to the fact that the drums were under lower tension than normal ones and also to their increase in mass due to the graft.

As the pieces of tissue used for grafts themselves lacked tension and as the natural tension of the tympanic membrane was destroyed when the perforations were produced, it would be expected that a drum restored by myringoplasty would not possess its normal tension As in the case of an atrophic drum scar such a postoperative slackness would be expected to impair the conditions for transmission of low-frequency sound The explanation of this dysfunction is probably that a drum with reduced tension is unable to vibrate as a rigid cone—a vibration pattern that is essential for the proper transmission of sound at frequencies below 2 400 Hz (v Békésy 1941 1960)

The increased mass of the grafted drums, on the other hand probably affected the transmission at higher frequencies,—a supposition that is borne out by the experimental series, where there appears to be a direct proportionality between the size of the graft and the impairment of high-frequency sound transmission Furthermore a comparison of the sensitivity curves in the respective test groups (figs. 20—22) with the threshold value curves calculated by Johansen (1948) on the basis of impedance formula discloses evidence of a parallelity or agreement with the curve that, according to the calculations, corresponds to an increase in the mass of the sound-conducting system Since the middle-ear transmission of sounds at frequencies higher than 2 400 Hz is influenced to a considerable degree by the elasticity in the incudostapedial joint (Møller 1965) it is conceivable that the increase in mas in the grafted drums reduces the elasticity in this joint with consequent impairment of the transmission of high frequencies.

Conclusion

In the experimental series the grafts of full thickness skin fascia and elastic cartilage—perichondrium tended slightly to impair the transmission properties of the middle ear this reduction was least pronouced at 1 000 Hz, slightly more at 200 Hz and most marked at 4 000—8 000 Hz.

The impairment of transmission was not so pronounced with the fascia as with the full thickness skin or the cartilage—perichondrium but the differences were very small

V Full Thickness Skin, Fascia and Cartilage with its Perichondrium in Myringoplasty

A summary and analysis of the results of 232 consecutive clinical operations.

Introduction

For the last 25 years the otosurgical treatment in the County of Norrbotten in the extreme north of Sweden has been centred at the Department of Otolaryngology of the Central Hospital in Boden (Head Gunnar Holmgren Jr M D) At this Department as in most other centres for middle ear surgery at the end of the 1950s only external body skin of full thickness were being used in myringoplasty Here as elsewhere, the unsatisfactory longterm results obtained with this graft tissue led to an increasing tendency to replace full thickness skin with various types of connective tissue Free autografts of fascia were thus commonly performed in 1962 When, however these too did not meet the perhaps, too high expectations placed on them, autogenous grafts of elastic cartilage with its perichondrium were introduced the following year as a conceivable alternative

Drum grafts containing cartilage were tested clinically by the author as early as 1961 but these were obtained from the hyaline skeleton of the nasal septum Owing to the difficulty of obtaining the required material from this site, since 1963 the cartilaginous graft has been taken from the elastic framework of the tragus—following Goodhill's (1961) favourable experience of tragal perichondrium in stapes surgery

The reason for including cartilage in these grafts was the supposition that the cartilage lamina fitted into the perforation might prove so substantially resistant to the poor nutrition at this site that closure through overgrowth from the surrounding host structures would occur even when the regenerative capacity of the vascular bed was low It was considered, moreover that this centrally positioned rigid plate would induce the restored membrane to vibrate lik the normal drum—that is, as a rigid piston—at lower frequencies.

So as to obtain a long term impression of the results of autogenous tympanic grafts of full-thickness external body skin, fascia and elastic cartilage with it perichondrium the present follow up study was undertaken As the object was to assess the actual drum graft with respect to its role in both anatomic and the functional restoration the only cases of interest were those in which, prior to operation, the drum defect had been judged to be solely responsible for any reduction in middle ear transmission

Material and methods

During the 5 year period from 1st July 1960 to 30th June 1965 a total of 241 myringoplasties (Wullstein type I tympanoplasties) were performed with autogenous grafts of full thickness external body skin fascia or elastic cartilage with its perichondrium This number does not include such myringoplasties performed on ears on which modified radical mastoidectomy had been carried out earlier or at the same seance

Of these 241 cases 232 could be examined with respect to the closure of the drum and the hearing restoration The follow up period was 3 1/2 years ± 12 months, unless a failure occurred before that

In the follow up series retroauricular full thickness skin was used on 79 occasions in 77 ears (2 re-operations) fascia on 74 occasions in 68 ears (6 re-operations) and elastic cartilage with its perichondrium on 79 occasions (0 re-operations)

Prior to operation the mean of the air bone gap at 500 1000 and 2000 Hz was on average 26 dB for the ears given skin grafts and 25 dB for those in which fascia or elastic cartilage—perichondrium was used

In the 9 myringoplasties the result of which could not be followed throughout the stipulated time owing to lack of co-operation by the patients, full thickness skin fascia and cartilage—perichondrium were used in 4 3 and 2 operations, respectively

All the drum perforations in the series were due to infection Two of the defects had been present for only 6 months, and the others for several years, some for decades.

The decision to perform myringoplasty was based on analysis of the ear before and at the actual operation

A preoperative microscopic examination and puretone audiometry were routine measures, while radiographic examination was seldom performed In about a third of the cases the transmissions properties of the middle ear were tested by the use of temporary drum membrane prosthesis The transmittance of the Eustachian tube was tested only roughly by Politzer's or Valsalva's manoeuvre

To minimize the danger of burying infection no operation was made on any ear that not had been dry the whole month prior to the myringoplasty

All the operations were performed in accordance with the generally accepted principles, insofar as these were applicable All were done by 4 qualified otologists Local anaesthesia was usually preferred

In the drum and middle ear examination and in the otosurgical manipulations the fine meato-tympanal structures were observed by means of a binocular magnifying glass (stereoscopically by Gullstrand's method) or an operation microscope (Zeiss Oto kop)

In the preparation of the graft bed any membrane remnants were carefully de-epithelialized and the perforation rim together with any parts of the lamina propria displaying tympanosclerotic changes were excised In the case of skin grafts in sub-total or total defects of the membrane the meatal epithelium was removed within a zone 5 mm from the annulus tympanicus and out into the auditory canal. When fascia or cartilage with perichondrium was used the corresponding canal wall skin was instead curled outwards before the adaption of the graft In the case of anteriorly located perforations the canal wall bulge in this region was removed with a diamond stone drill as proposed by Guilford *et al* (1959)

The full thickness skin grafts were taken from the retroauricular area. The transplants consisted of all the skin layers About one half of the grafts were of the pedicle type (Moritz s method 1952) and the other half free (Wullstein s method, 1953)

The fascia grafts were obtained as described by Örtegren (1959) from the superficial fascia of the temporal muscle and then from its ventral portion where the aponeurotic character is most prominent

The grafts of cartilage with perichondrium were taken from the elastic cartilage of the tragus by a technique similar to that described by Goodhill (1964) From the thumnail sized pieces of cartilage invested with connective tissue the perichondrium on one side was removed, while that on the opposite side was kept intact This perichondrium layer was made to overlap by about 2 mm the adherent cartilage lamina, which had been tailored to the size of the drum perforation

The greatest care was observed in manipulating the grafts. The skin grafts were placed over the perforation with the dermis portion against the de epithelialized meatal side of the bed The fascia grafts, which were likewise adapted, were covered on the canal side with a piece of oxidized regenerated cellulose (Surgicel® Johnson & Johnson) and, where feasible in the peripheral parts with the replaced canal wall skin The grafts of cartilage with perich ndrium were applied so that the cartilage lamina lay in the drum defect with the bare surface of the cartilage facing the middle-ear cavity The overlapping perichondrium on the opposite side then adhered to the denuded meatal structures surrounding the perforation. If possible the perichondrium portion was co ered peripherally by the pedicled meatal skin flap

After the operation the drum grafts were covered with a piece of gauze — dry for fascia transplants but otherwise moistened with a suspension of hydrocortisone and oxytetracycline (Terracortril® Pfizer) Gauze was als packed in the canal in such a way that the graft was only gently pressed against its bed.

Usually the patient was kept in hospital for 2 days after the operation The pack was remo ed after 7—10 days. Further checks were made on average 3 times during the first 6 months. If at the last of these examinations the status was satisfactory the patient was asked to report at intervals of

Of the 79 ears in which *retroauricular full thickness skin* was used 35 (44 per cent) showed an intact drum membrane Seven of these resembled the normal healthy tympanic membrane in 12 there was diffuse sclerosis under the ordinary shining meatal epithelium, and 16 consisted largely of typical external body skin Epithelial cysts were seen in 3 cases and in 10 the skin tran plants were red swollen and covered with desquamation products —manifestations of dermatitic processes

In 32 of the 44 ears where the graft had taken more or less incompletely or had later re perforated the perforation were partly surrounded by external body skin In 28 of these ears the transplanted skin was dermatitic in 5 small graft cholesteatoma were seen

Of the 74 ears in which a *fascia graft* was done 45 (61 per cent) showed complete closure of the drum perforation About one third of these drums resembled a quite normal tympanic membrane in one third the connective tissue stroma was to a varying extent thickened and opaque under the typical meatal epithelium and in the others this sclerosis was partly replaced by atrophy In 2 ears the intact drum membrane was the site of myringitis

In 10 of the 29 ears in which no closure was effected there was an extremely small perforation situated within an atrophic scar All but one of these 29 perforations were dry

Of the 79 ears in which grafts of *elastic cartilage with its perichondrium* were used the perforations were closed in 58 (73 per cent) at the end of the follow up period One of these drums looked like an ordinary tympanic membrane 2 displayed diffuse sclerosis, while the other 55 were characteriz ed by the grossly unchanged, sharply demarcated cartilage lamina In all these ears the restored drum membrane was covered with meatal epithelium which in one case was the site of myringitis. In 35 ears the incorporated cartilage graft was surrounded by a fairly normal membrane tissue In 20 there was some degree of atrophy in a zone of variable width adjacent to th cartilage lamina In 47 ears the cartilage graft was in close apposition to the adjacent tympanic membrane while in the other 8 the graft was partly dislocated In one ear the cartilaginous component was partly situated in side the restored membrane while in the other 7 ears some peripherial part of the graft deviated in the opposite direction giving rise to a local intumes cence on the meatal surface of the drum

In 17 of the 21 ears where the myringoplastic operation had failed the perforation was minimal and situated within an atrophic area just outsid the intact cartilage lamina In 4 ears the cartilaginous part of the transplant was missing and then the perforation was about the same size as originally All but one of these ears with persistent perforation were dry

Functional results

The results from the a pect f hearing r t ration are reported in figures 24 and 25

%

100

50

6 9 10 9

11 23 10

3 6 27 21

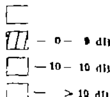
A B C

Fig. 24 Result of myri g plasty sessed with respect t the hearing rest rati g listered fter uccessful losure f tympa ic membra perforations. The result are pressed a the mean hift f l-conduction threshold at the frequ ncies 500, 1 000 and 2 000 Hz. The preoperati data re related t the res lt 2 1/2 years ± 12 month fter the peration

Column A — R troauricular f ll thickness skin (34 cases)
B — F scia (11 cases)
C — Cartilage with it perichondrium (57 cases)

Postoperativ rise in ir-conduction threshold —

lowering f air conduction threshold



— 0 – 9 dB
— 10 – 10 dB
— > 10 dB

× Numbe f ears i the respecti group

For full thickness skin grafts the mean lowering of the air-conduction threshold t 500 1000 an l 2000 Hz wa 10 dB or more in 56 per cent of the ears th rresponding figures for fascia and cartilage with perichondrium were 7a an l 81 per c nt respectively

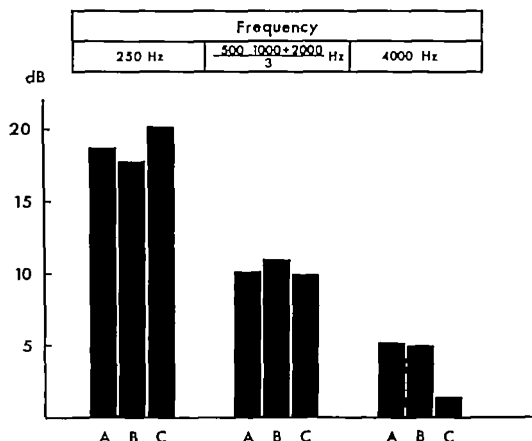


Fig 25 Result f m ri g pl st sessed with respect t the he ri g reai rati regist red fter uccessf l l re f tympa ic membran perforations. The mea reducti in dB, f the l -bone gap t 500 1 000 nd 2 000 Hz, d the reduction f the gap t 250 d 4 000 H re pressed the mea f r the ears grafted with the same type f ti The pre-operati e d t re rel ted t the re lt 3 1 2 ears ± 12 months fter the peration

Column A — B tron ricular f ll-thickness skin (31 cases)
B — Fasci (11 cases)
C — Cartilage with it perichondri m (37 cases)

At 250 Hz the reduction in the air-bone gap was on the average greatest for the ears with cartilage—perichondrium grafts (20 dB) the mean reduction of the gap at 500 1000 and 2000 Hz was on the average greatest for the ears with a fascia graft (11 dB) and at 4000 Hz it was on the average greatest for those with skin (5 dB) The range of the mean reduction of the gap for the 3 ear groups was comparatively small at the frequencies up to 2000 Hz, but at 4000 Hz it was nearly 4 dB this is because at this frequency the gap on average hardly diminished at all where cartilage was used

Discussion

To compare various tympanic graft tissues on the basis of the results of clinical myringoplasties it is necessary not only that the various materials shall have been applied under similar conditions and the assessment have been made consistently It is also necessary that each tissue shall have been used in series large enough for random negative results due to extremely poor restorative conditions to be fairly equally distributed between the various types.

These 3 conditions would appear to have been satisfied in the clinical material constituting the basis for the present comparisons between full thickness skin fascia and cartilage with its perichondrium Each tissue was used in more than 70 myringoplastic operations. In the 3 tissue groups the distribution of the perforations with respect to size was much the same This is indicated by the mean pre-operatively registered air bone gap at 500 1000 and 2000 Hz which, on average for the fascia and cartilage—perichondrium groups was the same (25 dB) and only 1 dB greater for the full-thickness skin group Throughout the time all these myringoplasties were performed the ENT Department in Boden was under the same head, thus increasing the chance of uniform assessment and management

The follow up period of 3 1/2 years ± 12 months was long enough to en ure that in no ear was the result of the operation regarded as successful unless it had remained so for at least 2 1/2 years. It is possible that the various grafts had integrated within 6 months, but it is not certain that their structural transformation will have been completed so soon In an assessment of the suitability of various tissues as drum grafts the state of the restored membranes after a few months is of minor relevance rather it is required to know whether the restored drums have tolerated the stress to which most tympanic membranes at times are subjected The observation time should thus be long enough for the reconstructed membranes to provide convincing evidence of their resistance—for instance in the case of upper respiratory tract infections or occasional barotrauma.

In reports of the results of variou clinical operations for hearing restoration the change in hearing obtained has usually been expressed as the difference between the pre- and postoperative air bone gap The reason for doing so is probably that the diastasis between the air and the bone-conduction threshold is regarded as a more reliable basis for comparisons than the threshold values themselves, which for a particular ear can vary from time to time—for instance from the point of the alertnes of the patient and through differences in the calibration of the audiometer

In the present context, however this does not apply entirely According to Oechl (1954) and Fournier (1959) the slightest impairment of the functional conditions in th middle ear not only leads to a rise of the air-conduction

threshold but is also reflected in a relatively small but definite rise in the bone-conduction threshold. If such a poorly functioning middle ear is restored the registered improvement in hearing will be a little better when expressed as the lowering of the air-conduction threshold than as the reduction of the air bone gap. Since, however, the graft tissues in this study were of structurally different types, it is likely that under the same conditions in identical ears they would, after integration, give rise to the same lowering of the air-conduction threshold but a different lowering of the corresponding bone-conduction threshold. This means that the drum grafts used here can hardly be correctly assessed from a functional standpoint solely on the basis of the difference between the pre- and postoperatively registered air bone gap.

The comparison of the 3 types of grafts was therefore made primarily on the basis of the surgically induced shifts in the air-conduction threshold, and only secondarily from the standpoint of changes in the air bone gap. Because of the comparatively large size of the test groups, whereby any measurement error would be distributed fairly evenly within the groups, this mode of procedure should be applicable in the present context.

Ideally, however, the comparisons should have been made on the basis of speech audiometry, but at the beginning of the present study this was not yet a standard method.

The results of the myringoplasties as regards the closure of the drum were best for cartilage with its perichondrium, slightly poorer for fascia and poorest for full thickness skin. Complete closure of the perforations was recorded in 73, 61 and 44 per cent of the ears, in the respective groups.

The overall relatively large number of failures is probably due to the fact that the only criterion of selection of the ears for the operations was that they should have been dry just the month prior to the operation, that the auditory tube should be passable by hyperbaric air, and that the ossicular chain should be intact. It would be extraordinary if such a series did not include a number of ears in which no restoration of the drum was possible—for instance because the tubal function was in fact insufficient, because cryptogenic infection had been overlooked, or because of extremely reduced mastoid cellularity, a factor that, according to Diamant (1965), tends to inhibit closure of ear-drum perforations.

The comparatively long follow up period and the length of the intervals between the examinations of the ears are other factors that might have influenced the results. Follow up periods of some years would be expected to increase the likelihood of drum injuries from, for instance, middle ear otitis or barotrauma, and in the long interval an incipient slough of a tympanic membrane graft afflicted by myringitis would be completed. Since a comparatively frequent follow up treatment is desirable after skin grafting in order that the subsequent desquamation products can be removed without delay, the fairly sporadic after-care in the present series would probably have affected the ears in which full thickness skin was used more than the

others. Thus, in a large series of such myringoplastic operations reported by Bandilow (1960) 82 per cent of the ears were dry after the operation when a frequent and regular examination had been carried out, against 34 per cent for patients only sporadically attending for inspection Since such frequent after-care is, from the patient's aspect neither encouraging nor desirable the mere necessity for it constitutes a disadvantage of external body skin in myringoplasty

As regards the improvement in hearing recorded post-operatively in the successful cases, there was no remarkable difference between the ears in which the various tissues were used

In the case of fascia and full thickness skin it is difficult to analyse the effect of the grafted tissues on the hearing, because the restored drum membranes thus obtained varied so greatly in shape—from completely normal ones to those bearing a close resemblance to the grafted tissue As it is impossible clearly to predict the post-operative appearance of any drum thus restored, the question of the acoustic properties of these 2 tissues is without practical significance In the case of cartilage with its perichondrium, however this was obviously not the case because in more than 90 per cent of the ears in which this tissue was used the cartilaginous part of the graft was grossly unchanged The crucial point in this connection will then be whether the sound transmission in the ears in which the drum consisted partly of a cartilage lamina was worse than in those to which either of the 2 other tissues had been grafted As regards the post-operative lowering of the air-conduction threshold it is then evident from the results of the audiometric tests that the ears with cartilage and perichondrium were slightly better than those of the other 2 groups If the reduction in the air bone gap is accepted as a suitable measure of the improvement in hearing, the above results show that the ears in which the missing drum tissue had been replaced by a cartilage graft were as good as, or slightly better than those of the other 2 groups at frequencies up to 2000 Hz, but at 4000 Hz perhaps slightly worse

Conclusions

It was found in this clinical study that permanent closure of the tympanic membrane perforations resulting from inflammation was obtained more often after the use of tympanic grafts of autogenous cartilage with its perichondrium than of those of autogenous fascia, and more often after the use of fascia than autogenous external body skin of full-thickness. The closure of the perforations with the 3 graft tissues resulted in, on average approximately the same mean reduction in the air bone gap at the frequencies 500 1000 and 2000 Hz

of the graft survive the transfer If this cell survival theory is generally valid and the grafted connective tissue cells have in fact survived the change of site the drum closed with a fascia graft should have the typical appearance of aponeurotic connective tissue In the present experimental series, where the host site was supposed to be well vascularized this was the case for only one fascia graft In the corresponding clinical material too there was no membrane that truly resembled fascia but instead most of the post-operatively closed drum membranes displayed only sparse and diffuse sclerosis.

Another interesting feature of these human drums, however was the more or less extensive atrophy that was often observed in the restored membranes. When re-perforation occurred the perforation was almost invariably found within these atrophic areas

These observations would suggest that in myringoplasty the fascia graft does not readily integrate with the bed with a retained viability If this is the case the drums repaired with fascia and reported as beeing healed would be largely composed not of the graft tissue but of regenerated elements from the bed Since the regenerative power of the human body tends to diminish with age the above view would seem to be borne out by Örtegren s (1964) observation that myringoplasty with fascia is less often successful above than below 40 years of age

As fascia and tendon tissues are morphologically closely related it is perhaps possible in this connection to draw a parallel between a gliding tendon graft placed in interposition and a fascia graft approximated over a drum perforation for the vascular beds are just as incomplete in the two cases. As regards the integration of such a free tendon graft Heinemann (1963) found that the transplant usually succumbs and is substituted completely by connective tissue deriving from the bed

As the oxygen needs of full thickness skin per unit of volume are about twice that of connective tissue (Krebs et al., 1948) it might appear curious that the full thickness skin grafts of the present experimental series survived transfer to the drum bed better than the fascia grafts, which underwent marked changes A possible explanation is to be found in the different vascular pattern in the two tissues. In the fascia the vessels lie in a plane which is coincident with the surface of the underlying muscles while in the skin they course for a part perpendicular to the surface This means that many more transected capillary vessels are directed towards the opened vessels of the bed in a skin than in a fascia graft

If however fascia is less fitted to survive the transfer to the drum remnants than external body skin it might appear curious that the fascia grafts in the present clinical series yielded considerably better results than the corresponding skin grafts in respect of closure of the tympanic membrane The explanation would then lie in the completely different reaction of these two tissues in closure of the perforation Here the fascia graft is obviously less involved than the skin graft, which, while replacing the

original defect at the same time actively inhibits any natural regeneration of the drum tissue The fascia tissue on the other hand, probably supports passively such regeneration in serving as a scaffold for the proliferating connective tissue cells of the bed, and as a vector for the both meatally and tympanically regenerating host integumentary cells. Hence if the vascular bed is more or less unfavourable for both these grafts the course of healing should favour the fascia grafted drum After fascia grafting the subsequent degeneration of the transplant will take place many times under cover of invaded host epithelial cells. If then the regenerative capacity of the bed is sufficient to produce a substantial connective tissue replacement, a membrane of appropriate thickness will be obtained otherwise a more or less atrophic drum will result The corresponding degeneration of a skin graft, however starts, within the epithelial portion When these structures degenerate and die they are unlike the connective tissue in the fascia graft completely unprotected from attacks by the numerous microorganisms normally present on the surface of the skin. This combination of degenerative and inflammatory changes will in the long run, result in perforations in a great number of cases.

It is thus tempting to conclude that a full skin graft by incorporation as such prejudices the permanent closure more than a fascia graft which disappears during the healing process.

Cartilage—perichondrium

When the pieces of elastic cartilage with adherent perichondrium had been transplanted to the well vascularized beds in the cat ears the cartilage integrated without any gross changes Histologic examination showed the corresponding chondrocytes to be largely vital The parts of the perichondrium that initially overlapped the cartilage, on the other hand, had greatly diminished during healing In the corresponding clinical series, too, the cartilage almost invariably lay macroscopically unchanged within the host site Several of these human transplants, however were situated in membranes that were more or less atrophic next to the cartilage when there was a perforation it was almost invariably situated within such an area

From the above observations it would seem that the cartilage component of these grafts had a greater capacity than either full thickness skin or fascia to survive intact the transfer to the incomplete vascular bed of a perforated drum. This superior power of the cartilage is probably due to this tissue s nutrition by diffusion and its low oxygen consumption

As regards the behaviour of these transplants in closure of drum perforations, it would appear that the integrated cartilage component, like a skin graft, can in fact replace the tissue defect in the drum but that the cartilage unlike external body skin, instead tends to promote natural regeneration The cartilage lamina acts in this sense as a neutral but vital substratum for

the proliferating host tissue cells that envelope the graft Even in the case of a relatively low regenerative power of the bed this would mean that there is a good chance that closure of the perforation will occur not with an atrophic scar but with a cartilage armed, and hence slightly thickened membrane On the other hand the perichondrium component the purpose of which in this context is to anchor the cartilage probably takes the same part in the closure as was ascribed earlier to the fascia. In consequence myringoplasty with grafts of cartilage and perichondrium probably will give a higher proportion closures provided that the cartilage lamina is tailored to fill the perforation completely

Acoustic restoration—Although the tissue grafts of elastic cartilage with its perichondrium proved to be slightly better as regards drum closure than those of fascia, and decidedly better than those of full thickness skin in an evaluation of the suitability of these tissues for drum grafts it is necessary also to know whether the definitive restorations obtained with them differ appreciably in acoustic quality—that is, as regards their effect on the transmission of sound To examine this the transmission properties of the middle ears were determined in the experimental and clinical series. In the cats this was done by comparing the grafted ears with ears not operated upon on the basis of a predetermined cochlear microphonic response In the patients, pure tone audiometry was used These two studies disclosed no essential difference between the 3 groups as regards the capacity of the middle ear to transmit sound at the speech frequencies. At lower and higher frequencies, however there was probably a slight difference Low frequency sound was transmitted a little better in the ears of the clinical series with cartilage—perchondrium grafts, while at 4000 Hz the opposite was the case for both the experimental and clinical series As mentioned in chapter IV these small differences might be due to the comparatively greater rigidity and mass of the cartilage graft.

VII General Summary

The object of this investigation was to compare autogenous full-thickness skin, fascia and elastic cartilage—perichondrium as materials for tympanic grafts Of principal interest was their capacity of restoring the perforated drum, and, secondly the acoustic quality of the membranes obtained

So that these comparisons might be made on a wider basis than is usual, studies were performed experimentally in laboratory animals as well as in man In the experimental series the conditions for transplantation were considered to be favourable and in the clinical series less so

Material and methods

The experimental study was performed on the cat. Freshly produced perforations of the ear-drum were closed with grafts of full-thickness skin (10 ears) fascia (10 ears) and elastic cartilage—perichondrium (11 ears) After a mean follow up period of just less than 6 months the healed drums were examined as regards function and, after the animals had been sacrificed, from the morphologic aspect. In the functional evaluation a comparison with respect to the middle-ear transmission properties was made with an average ear in which no graft had been performed. The point of reference then used was a cochlear microphonic response of 3.5 µV RMS, recorded at the round window In the morphologic evaluatioin the sectioned temporal bone was examined histologically

The clinical study consisted in a follow up investigation of a consecutive clinical series of myringoplasties. Grafts of retroauricular full thickness skin were used on 79 occasions, fascia on 74 and elastic cartilage—perichondrium on 79 The follow up period for the series was 31/2 years ± 12 months.

Results

Experimental study

(1) *Full-thickness skin* — The grafts were integrated and retained their chara ter of full skin Glands, hair follicles and proliferations of the squamous epithelium caused the formati n f multiple epithelial cysts. No dermatitic processes were observed

(*Fa cia* – With one excepti n the grafts were reduced to, or replaced by a parse atypical connecti e ti ue In the exceptional case the graft was g ly and microscopically unchanged.

(3) *Cartilage—perichondrium* — The cartilage component of the grafts was usually unchanged but the perichondrium portion like fascia was reduced to or replaced by a non specific connective tissue

(4) The middle-ear sound transmission was, on average slightly poorer in the experimental than in the normal" ear a difference that was least marked at 1000 Hz, slightly more so at 200 Hz, and most pronounced at frequencies above 3000 Hz. The transmission properties was slightly better in the ears with a fascia graft than in those where the other tissues had been used

Clinical study

(1)—In the ears with full thickness skin grafts structures with the character of skin were rarely to be seen and when such structures were observed they usually displayed dermatitis —In the ears with fascia grafts there was no typical fascia tissue Instead there were irregular sclerotic areas and atrophic scars of varied extent —In the ears with grafts of elastic cartilage—perichondrium the cartilage component was almost invariably present in a grossly unchanged state the perichondrium on the other hand, had the appearance of integrated fascia

(2) Of the ears with grafts of retroauricular full thickness skin 44 per cent of the perforations were closed For fascia and elastic cartilage—perichondrium the corresponding figures were 61 and 73 per cent

(3) In 56 per cent of the ears in which the full skin transplantations resulted in closure of the perforation the air-conduction threshold was reduced by more than 0 dB (the mean value for tones of 500 1000 and 2000 Hz) For fascia and cartilage—perichondrium the corresponding figures were 75 and 84 per cent

Conclusions

The results of the study suggest the following conclusions.

(a) Drum grafts of autogenous fascia and cartilage—perichondrium are superior to those of full thickness external body skin

(b) In ears in which the drum remnant is rendered fibrotic and regeneratively insufficient autogenous cartilage—perichondrium is probably more suitable than fascia as a material for drum grafts.

Zusammenfassung

Die vorliegende Untersuchung hatte zur Aufgabe, die Anwendbarkeit von autogenem Gewebetransplantat aus Vollhaut, Faszie und elastischem Knorpel mit dazugehörendem Perichondrium als Material bei der Myringoplastik zu untersuchen und zu vergleichen. Es interessierte sowohl die Möglichkeit der Defektdeckung am Trommelfell mit dem genannten Gewebematerial als auch die akustischen Eigenschaften der dabei gebildeten Membranen zu studieren.

Die verschiedenen Arten der Transplantate wurden tierexperimentell und klinisch untersucht. Den Vergleichen sollte dadurch eine etwas breitere Basis verliehen werden. Die Bedingungen eines erfolgreichen Einheilens wurden in dem experimentellen Material als günstiger angesehen verglichen mit dem klinischen Material auf grund besserer zirkulatorischer Bedingungen.

Material und Methode

Die tierexperimentellen Studien wurden an Katzen vorgenommen. Zentrale frische traumatische Trommelfellperforationen wurden mit Vollhaut (10 Ohren), Faszie (10 Ohren) und elastischem Knorpel-Perichondrium (11 Ohren) gedeckt. Nach einer durchschnittlichen Beobachtungszeit von ungefähr 6 Monaten wurden die geiheilten Trommelfelle funktionell und nach Tötung der Tiere ebenfalls morphologisch untersucht.

Funktionell wurden die myringoplastisch operierten Ohren in Hinsicht auf die Mittelohrtransmission verglichen mit einem nicht operierten „Durchschnitts-Ohr", wobei als Referenz ein Microphonopotential der Schnecke von 3,5 μV RMS, abgeleitet vom runden Fenster angewandt wurde. — Die morphologische Untersuchung der Membranen wurde an den zu histologischen Präparaten verwandelten Temporalknochen vorgenommen.

Die klinischen Studien umfassten Nachuntersuchungen eines konsekutiv myringoplastisch operierten Patientenguts. In diesem war retroauriculäre Vollhaut in 79 Fällen, Faszie in 74 und elastisches Knorpel-Perichondrium in 79 Fällen zur Anwendung gekommen. Die postoperative Beobachtung betrug 3 1/2 Jahre ± 12 Monate.

Ergebnisse

A. Experimentelle

1. *Vollhaut* — die Transplantate hatten hauptsächlich gleichen Charakter wie die Haut der Entnahmestelle, sie enthielten Haare und Drüsen. Im Chorium fand man zahlreiche Zysten, jedoch keine entzündlichen Prozesse.

2 *Faszie* — die Transplantate waren bis auf eine Ausnahme reduziert zu oder ersetzt durch ein sparsames, uncharakteristisches Bindegewebe In dem einzigen Ausnahmefall war das Faszientransplantat sowohl makroskopisch wie mikroskopisch unverändert

3 *Knorpel Perichondrium* — die Knorpelkomponente der Transplantate waren hauptsächlich unverändert der Anteil des Perichondrium dagegen reduziert oder durch Bindegewebe ersetzt

4 *Mittelohrtransmission* — allgemein etwas schlechter in den experimentellen Ohren als im „Normalohr" Der geringste Unterschied bestand bei der Frequenz von 1000 Hz, etwas stärker merkbar bei 200 Hz und am meisten ausgeprägt bei Frequenzen die 3000 Hz übersteigen Das Transmissionsvermögen war etwas besser bei den mit Fasziegewebe operierten Ohren als in den übrigen Experimentohren

B Klinische

1 — In den mit *Vollhauttransplantat* operierten Ohren wurden verhältnismässig selten epidermale Strukturen beobachtet, und falls vorhanden waren diese myringitisch verandert — In den mit *Faszientransplantat* operierten Ohren konnte man niemals eine typische Faszienstruktur nachweisen stattdessen fand man in variierendem Ausmass sklerotische Streifen und atrophische Narben — In den mit *Knorpel Perichondrium* operierten Ohren sah man fast immer die Knorpelkomponente die makroskopisch unverandert erschien In dem Teil des Einheilungsproduktes, der dem überschiessenden Perichondrium entsprach sah man dagegen in wechselndem Ausmass nur Sklerose und Atrophie

2 In den mit retroaurikulärer Vollhauttransplantat operierten Ohren waren 44 % der Perforationen geschlossen Für Fasziengewebe und elastischem Knorpel Perichondrium waren die entsprechenden Ziffern 61 % respektive 73 %

3 In 56 % der Ohren in denen die Vollhauttransplantation zur Ausheilung der Perforation führte war die Hörschwelle für luftübertragenen Ton mit mehr als 9 dB gesenkt (Mittelwert für die Frequenzen 500 1000 und 2000 Hz) Für die mit Fasziengewebe und Knorpel Perichondrium operierten Ohren waren die entsprechenden Ziffern 75 % respektive 84 %

Konklusion

Als Resultat der Untersuchungen ergibt sich

1 dass autogene Trommelfelltransplantate mit Faszlengewebe oder Knorpel Perichondrium Vollhauttransplantaten überlegen sind

2. dass autogene Trommelfelltransplantate mit Knorpel Perichondrium vermutlich Transplantaten mit Fasziengewebe in solchen Ohren überlegen sind in denen die Trommelfellreste fibrotisch und regenerativ insuffizient sind

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T PALVA and K DAMMERT

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T. PALVA and K. DAMMERT

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CONTENTS

I INTRODUCTION

The connections between the labyrinthine fluids and the cerebrospinal fluid (CSF) space have stimulated the interest of otologists for a long time. Since 1774 when Cotugno described a narrow passage passing from the scala tympani to the CSF space, the cochlear aqueduct has been considered the main anatomical pathway between the two fluids. In the early part of the 20th century the thorough studies of Karlefors, Meurman and Karbowski in particular supplied much detailed new knowledge on this canal, and recently there have been a number of studies connected with the efforts to determine the origin of the labyrinthine fluids.

Nevertheless, as will appear from the following review there are still a number of controversial questions, and the patency of the aqueduct, in particular has remained in doubt.

Some of the reasons for the divergent results appear to be due to technical details in sectioning of the specimens. If the sections are cut across the main course of the aqueduct — a procedure mainly used earlier — a correct idea may be formed of some aqueduct dimensions. However misleading opinions may be formed particularly on the aqueduct patency if only every 5th or 10th section is studied, while the slope of the aqueduct is not straight.

We intend here to evaluate the anatomy of the cochlear aqueduct mainly on the basis of sections cut parallel to the slope of the aqueduct for its whole length and studied one by one. By this means it is also possible to keep the number of sections per specimen within reasonable limits, and a reliable basis should be found for evaluation of various details of the aqueduct and the surrounding structures.

II REVIEW OF EARLIER STUDIES

Karlefors, in 1924 started his study on the cochlear aqueduct by first treating a 13.5 cm long human embryo with a dye solution injected through the round window into the scala tympani Dye from the labyrinths was found in histologic sections in the aqueduct in the CSF space, particularly around the glossopharyngeal nerve, but not in the meatus acusticus internus. However it also appeared around the aqueduct and in the surrounding marrow spaces Since only little dye was seen in the aqueduct, it was thought that the main amount might have passed by a route other than the aqueduct Karlefors emphasized the importance of histological studies stating that passage of the fluid into the CSF space does not necessarily imply patency of the duct.

The studies were then continued using fifteen embryos and fetuses, from 28 8 mm crown rump length to the mature fetus, without preliminary in jections of dye The canal was found to start developing in 53—56 mm embryos beginning from the scala tympani it opened in the subarachnoid space. The loose connective tissue in the canal was considered a direct extension of the arachnoid. Close to the internal opening at the scala tympani the canal generally was filled with loose connective tissue The cochlear vein was found near the internal orifice of the aqueduct and it was then running parallel to the canal and in front of it, ending in the inferior petrosal sinus

For further elucidation of whether the aqueduct is open in adult ears or not, the vertical semicircular canal was opened in 4 cadaver ears and a suction tip employed at this place while dye solution was placed at the external opening of the cochlear aqueduct. In two ears dye solution filled the aqueduct and was seen in the cochlea one of the specimens showed similar filling in the case of the aqueduct and the vein canal. In the fourth ear no dye entered the cochlea after suctioning of the vertical canal.

Karlefors performed further histological studies on 14 temporal bones, subjects aged eight months to seventy years. He concluded that both the subdural and subarachnoid space continue in the aqueduct the subdural space varied widely being sometimes nearly absent and sometimes of considerable size for the entire course of the aqueduct As a rule arachnoid tissue appeared inside the canal and the subarachnoid space could vary in large limits. Along the course of the aqueduct the subarachnoid space was found to be at its minimum about 1 mm from the scala tympani, where it was filled with loose connective tissue, the internal opening being again wider The length of the canal was measured in two adult cases, and was 11 mm in one, 15 mm in the other The distance from the aqueduct to the parallel cochlear vein channel was about one mm

Karlefors thought that the connective tissue in the subarachnoid space near the scala tympani caused an occlusion of the whole duct leaving no free passage for fluid from the CSF space into the cochlea. In children with a wider canal the fluid appeared to pass through more easily

A few years later Meurman (1930) studied various aspects of the cochlear aqueduct in 55 temporal bones from 32 persons. Similarly as Karlefors, he cut most of the sections across the aqueduct only one temporal bone was cut parallel to it. The canal was found to pass through compact bone but was often surrounded by pneumatic or marrow spaces. Its cochlear orifice was generally situated 0.3 mm from the round window membrane. In one case with a high jugular bulb the canal was short and seemed to adjoin the bulb without any separating bony canal.

Because the sections were cut across the canal estimation of its length was uncertain. In one newborn infant, the canal was about 5 mm long and was found to grow longer with age. The general adult length according to Meurman was 10—15 mm, which included the dural cover of the canal. The width of the canal was measured at various points the narrowest area was about 500 μ from the cochlear orifice average width being 90 μ from endosteum to endosteum and 110 μ from bone to bone. Average width 1 mm from the cochlea was the same, but thereafter increased, so that 4 mm from the cochlea the bony canal had a width of 420 μ. The diameter increased further: 6 mm from the cochlea it was 920 μ, and over 8 mm from the scala tympani as much as 1650 μ. Corresponding to this increase of width, the soft tissue inside the canal grew in thickness 0.5 to 1 mm away from the cochlea it measured 10 to 20 μ but 7 to 8 mm from the cochlea it reached the figure 205 to 230 μ.

Meurman claimed that in four of his cases the canal was totally obliterated for a short distance This he thought was due to a developmental disorder or to endosteal (periosteal) bone proliferation during life particularly in old age Perusal of his text suggests, however that there was total obliteration in none and that, possibly the sections did not represent the area of the aqueduct. He also noticed that in some cases small bony exostoses stuck into the canal lumen.

In children the canal had about the same size as in adults because it was short, it was relatively wider

The greater part of the bony canal lumen in certain areas seemed to be filled by the arachnoid tube, starting from the glossopharyngeal nerve in some cases, on the other hand, there was a wide space between the arachnoid lining and the periost bone. However Meurman had the astute thought that this subdural slit in cadavers may be artificial and absent during life .He also supported Karlefors in stating that 1 to 2 mm from the cochlea the arachnoid tube was often very dense sometimes one could not find any lumen. In 11 cases, 0.5 mm from the scala tympani, the arachnoid lumen was found to be occluded in 7 loosely filled in 3 and open in one only One mm from the scala tympani the lumen was closed in all except one

The corpora amylacea first noted in the cochlear aqueduct by Habermann

(1882) were also noted by Meurman, mostly at the borderline between the arachnoid and dura, sometimes in the dura and in the lumen of the canal. They varied in form, being sometimes round or oval, sometimes longitudinal Meurman speculated that they might originate from an atrophic part of a blood vessel: this particle then collected extra material on its surface and assumed various forms.

The cochlear vein channel was reported by Meurman to run 1/2 to 2 mm away from the aqueduct. The vein ended in the inferior petrosal sinus or directly in the jugular bulb In some cases it ran in the same canal as the aqueduct, but was always separated from it by the dura. It was of same width as the aqueduct in the middle part, but somewhat larger near the scala tympani

The third investigator of that time, Karbowski (1930) noted that the arachnoid lining in the aqueduct was as a rule adherent to the dura. The arachnoid lumen often contained a loose netlike connective tissue, which could vary in size and sometimes filled the channel wholly The duct was found to be open in only 3 out of 14 cases, and in these three there was no additional tissue in the arachnoid tube Karbowski also noticed deep-blue longitudinal or roundish particles in the walls of the channel — the corpora amylacea of Meurman — but did not observe the subdural open spaces described by the latter

Karbowski injected Chinese ink into the suboccipital space, and demonstrated it in the scala tympani and in the aqueduct proper sometimes in large amounts. He concluded that, in the cases in which the aqueduct is open it clearly functions as a pathway for fluid circulation. In agreement with Karlefors, he found that ink appeared in the air cells and also in the sub-epithelial tissue close to the veins in the lower part of the middle ear

Of recent writers Waltner (1947) studied a material of 70 temporal bones (age from 3 days to 86 years) for corpora amylacea. The amylaceous bodies were on average 0 03 mm in diameter (range 0.01 to 0 1 mm) and elongated or elliptic in shape. Stained with hematoxylin-eosin, some seemed to appear structureless, some showed three to sixteen concentric rings, the colour alternating from light to deep blue. The rings were separated from each other by dark cement lines With iodine stain all corpora amylacea stained deep brown since the calcium salts are removed in the process of decalcification what remains is probably starchy material.

Waltner noted that the corpora amylacea apperared from the age of six upwards. They were seen in the arachnoidea and dura surrounding the glossopharyngeal nerve in all cases in which the sections contained the region of the ganglion at the inferior opening of the cochlear aqueduct. In general the bones of old people contained more corpora amylacea than those of the young, but there were numerous exceptions.

Waltner considered corpora amylacea normal landmarks of the cochlear aqueduct their absence being associated with the absence of the arachnoid reticulum pulled out during removal of the petrous bone from the skull. There were no clear connections between the corpora amylacea and any disease,

and the bodies were distributed along the entire course of the cochlear aqueduct and sometimes within the scala tympani at the base of the cochlea.

In studying the histogenesis Waltner found that one or more ghost cells of the arachnoid type may be visible in the center of many of the amylaceous bodies. Arachnoid like cell clusters were frequently present side by side with corpora amylacea. The cells had large nuclei they were closely packed round, with more or less recognizable cell outlines. The fact that all these stages up to the fully developed corpora amylacea were found in several specimens and that they were never found in fetuses or bones before the age of three, pointed strongly against the claim that the corpora amylacea are artefacts.

Besides in the aqueduct, corpora amylacea occurred in the meninges of the seventh, eight and ninth cranial nerves. Waltner postulated that the same circulating substance reaches the arachnoid cells in various parts of the skull and participates in the infiltrating process of the arachnoid cell clusters. Corpora amylacea were never found around the cochlear vein — a fact suggesting that the substance originates from the spinal fluid rather than from the blood The aqueduct was never completely obliterated by corpora amylacea, although in the narrow portion 1—2 mm from the cochlea, a marked narrowing of the lumen was often seen

Waltner called attention to the fact that the aqueduct is never a fully open canal the narrow part of the aqueduct at the distance of 1—2.5 mm from the scala tympani and the ever present reticulum prevent any rapid flow of the spinal fluid. The only physiologic role of the corpora amylacea might consist in further reducing the lumen of the aqueduct and slowing down the flow of the spinal fluid.

In another paper Waltner (1948) described a barrier membrane situated in the internal opening of the aqueduct. This membrane seemed to prevent the flow of erythrocytes or pus cells from CSF space into the perilymphatic space. He claimed that the membrane is present in 11 cm long fetuses, at which stage it consists of two to three layers of arachnoid type cells one end of the separating membrane adjoins the endosteum of the scala tympani directly and the other the inner surface of the round window membrane In adults the barrier membrane is a direct continuation of the neighbouring endosteum it consists of a single layer of cells and measures 1 micron or less in thickness. In some instances the reticulum of the aqueduct was in contact with the barrier membrane, in others it was clearly separated by an empty space. Waltner's conclusion was that under physiologic conditions a diffusion but no direct flow occurs between the spinal fluid and perilymph through the barrier membrane.

Anson et al. (1964 1965) studied the cochlear aqueduct in 10 temporal bones from adults and found the average diameter of the internal aperture of the cochlear aqueduct to be 0.09 mm with a range of 0.05 to 0.125 mm. The average dimension of the external aperture was 0.83 mm (range 0.5 — 1.25 mm) These measurements were made 1 to 2 mm inside the funnel-shaped opening of the aqueduct on the bone surface. In the same specimens the

average length of the aqueduct was 11.5 mm (range 10.0 — 14 0 mm) Prominent vessels were lacking in the aqueduct, which contained connective tissue The cochlear vein was found to occupy a separate osseous channel adjacent to the aqueduct. The final conclusion of these investigators was that the cochlear aqueduct is filled by tissue through which fluids generally pass in the body

Contrasting results were reported in the same year (1965) by Ritter and Lawrence In five human temporal bones the length of the aqueduct varied from 5.2 — 7 4 mm (average 6 45 mm) — about one half of the length previously reported. The width was measured in 19 temporal bones at three points the entrance of the aqueduct into the scala tympani, its exit from the temporal bone, and approximately midway between these two As the lumen did not always contain the periotic duct the distance between the bony walls only was recorded. Contrary to earlier data, the aqueduct was most narrow in its middle part and thus had an hourglass shape. The diameter at the opening into the scala tympani in 19 specimens averaged 0 1 mm, at the mid point 0 09 mm and at the exit from the temporal bone 1.3 mm.

In 9 temporal bones the canal contained soft tissue which in some sections had the appearance of a solid core of tissue filling the aqueduct, while in others there was merely a loose syncytium In no specimen could a layer of cells be identified across the internal orifice of the aqueduct as recorded by Waltner

In 17 patients subjected to stapedectomy 2 cc of indigo carmin was instilled into the CSF space prior to surgery during the operation perilymph was collected from the oval window and examined for the dye. Despite the fact that the patients were asked to lie with the ear undermost in a Trendelenburg position, no dye was ever seen in the perilymph. A second lumbar puncture was therefore done on one patient 24 hours later and the fluid was a brilliant blue colour In another patient, a lumbar puncture 74 hours after injection showed clear fluid. Ritter and Lawrence concluded that connective tissue fills the cochlear aqueduct, which is not sufficiently open to permit a free fluid flow

In addition to studies on the normal structure of the cochlear aqueduct, reports are available on the patency of the aqueduct in diseases. One of the most extensive papers, by Growe (1930) describes a series of cases of meningitis in relation to changes in the internal ear It includes a child aged 4 months with a widely open cochlear aqueduct. This was lined throughout with dura and the lumen was filled with arachnoid tissue which continued and merged with that covering the cerebellar lobes. Another patient, aged 9 1/2 years, had streptococcic meningitis and labyrinthitis the open cochlear aqueduct showed a typical infected appearance with a destroyed arachnoid.

Similar observations of a free passage through the cochlear aqueduct were made in patients who had succumbed to an intracranial operation for brain tumour One patient, aged 31 years, showed blood in the aqueduct and in the basal coil of the scala tympani on both sides Crowe assumed that blood did not

gravitate into the cochlea after death or during the manipulations at autopsy and this was proved by the fact that, during a five year period, the removed temporal bones did not reveal erythrocytes in the scala tympani unless there was infection, leucemia, or an operative or traumatic skull injury

In cases of subarachnoid hemorrhage Perlman and Lindsay (1939) also found red blood cells in the scala tympani at the mouth of the aqueduct. These observations suggested a fluid flow though a slow one, from the subarachnoid space to the cochlea. This opinion was corroborated by the finding that, in cases in which the cochlea had been experimentally injured to produce bleeding within the perilymphatic spaces, there was no tendency for the blood cells to collect in the aqueduct region

Recently Henneford and Lindsay (1968) reported on 2 patients who lost their hearing at the ages of one and two years apparently due to meningitis. The probable pathway of infection was the cochlear aqueduct although, in the latter case at autopsy in old age it was obliterated by bone at its opening into the scala tympani. Holden and Schuknecht's (1968) series of 12 ears with spontaneous subarachnoid hemorrhage showed that the cochlear aqueducts of 6 ears were large enough to admit erythrocytes into the scala tympani. Blood had entered the internal auditory meatus in all ears and had extended into Rosenthal's canal in 7 ears and the osseous spiral lamina in 3 These last three had small cochlear aqueducts suggesting that circulation between the cerebrospinal fluid and perilymph occurred through the internal auditory meatus and modiolus. There was no evidence, however that blood cells could enter the scalae of the inner ear through the modiolus.

To sum up in the studies reported above there is a clear difference of opinion as to whether the cochlear aqueduct allows a free passage of fluid from the CSF space into the scala tympani Of the early writers, Karlefors held the opinion that in children with a wider canal the fluid passes through it, but in adults there is no exchange of fluids. Meurman stated that there is no difference in width between children and adults but, since the former have a shorter canal, it is relatively wider Both he and Karlefors doubted the free interchange of fluids in Meurman's view the arachnoid tube appeared blocked in nearly all cases 1 mm from the scala tympani. In Karbowski's series most of the temporal bones had a closed aqueduct it was fully open in 3 out of 14 On the other hand, the suction experiments of Karlefors and the instillation of dye into the CSF space (Karbowski) are in favour of the cochlear aqueduct being an open fluid exchange pathway

The observations made by Crowe and by Perlman and Lindsay after lethal meningitis or subarachnoid hemorrhage are arguments for an open cochlear aqueduct. Crowe explained the fact that meningitis in adults only seldom causes labyrinthitis and deafness by stating that, in these longer canals, the arachnoid tissue is capable of totally filling the aqueduct lumen due to infectious swelling and so protects against the spread of infection by this route.

Of recent writers, Anson et al. are convinced that fluid easily passes

IV RESULTS

A THE BONY AQUEDUCT

Before starting decalcification of the temporal bones, an attempt was made in the 15 cases with removed round window membrane to determine whether or not the cochlear aqueduct is open to injected fluids. For this purpose an eosin dye solution was injected with a blunt-tipped needle into the external orifice of the aqueduct. This was done before any trimming of the specimens when the soft tissues were still intact No force was used in these experiments to avoid the possible false routes demonstrated by Karlefors. When the internal orifice of the cochlear aqueduct was examined by loupe magnification eosin solution was seen to enter the scala tympani in 6 specimens during injection under gentle pressure. In 9 specimens no dye entered the scala tympani and an immediate resistance was felt on attempted injection

Though it was endeavoured to section the cochlear aqueduct parallel to its course and thus demonstrate it from the external orifice up to the opening in the scala tympani, this succeeded in none of the specimens. In the majority of cases it was possible to demonstrate the whole aqueduct in a few consecutive sections mostly about half of it was seen in one section This was in part because the sections were not exactly parallel, and in part because the course of the aqueduct itself is not straight, but its slope can include even a considerable curve. By studying each consecutive section however a clear idea emerged about the structure and patency of the aqueduct.

The measurements and the observations on aqueduct anatomy are presented in Table I The length was measured from the cochlear orifice of the aqueduct through the bone to the point where a far larger funnel-shaped depression begins to form on the inferior surface of the petrous pyramid. In most instances the length of the canal of the aqueduct proper was clearly definable in the consecutive sections. Examples of these measurements in parallel sections are seen in Figs. 1 to 3 Fig. 1 shows that the aqueduct gradually changes into a large funnel without a sharp borderline at the external orifice. The use of a section 200 μ away from this level (Fig. 2) gives additional information about the external orifice by defining the bottom of the channel just outside the aqueduct lumen Fig 3 shows more than half of the aqueduct (except its external orifice), exemplifying the majority of cases with a slightly oblique course of aqueduct (or section) in which the length could be measured exactly from one section.

TABLE I

Dimensions of cochlear aqueduct

Bone No	Length (mm)	Width: Internal Orifice	(w) at various points (mm) from scala tympani: 0.5	1	1.5	2	3	4	5	6	7	8	Funnel
1	5.7	300	180	150	190	390	340	570	990				1900
2	6.5	450	180	210	220	330	420	480	690	1020			2100
3	6	270	150	210	300	350	420	450	360	510			690
4	7	210	90	80	90	90	150	120	120	480			900
5	8	230×390	150×310		150×390	160×410		390×600			410×900		1500
6	8	250	160	150	160	190	270	350	550	600	700	780	2400
7	6.5	400	210	140	180	240	300	360	630	690			2700
8	7	350	180	120	150	160	190	240	240	290	690		2300
9	7	210	150	150	150	180	300	390	460	580	700		1800
10	7	210	150	150	210	280	320	780	1500	2400			3000
11	6	210	150	160	210	260	300	350	410	800			2100
12	5	310×400	150×290		300×400	360×460		310×360					1800
13	6	300	120	125	120	180	240	300	600	1100			2400
14	5	270	180	120	150	180	180	} One wall mum g					
15	6	280	190	130	150	170	200						
16	6	450	120	120	150	210	220	270	330	360			1200
17	5	600	180	110	120	150	170	190	300				750
18	5	450	150	170	200	210×750		300×1050					1900
19	6	300	120	190	150	150×630		210×630		1990×1300			1500
20	6	300	120	120	150	210	330	450	1010				1300
Mean	6.2	322	151	145	175	231	276	371					

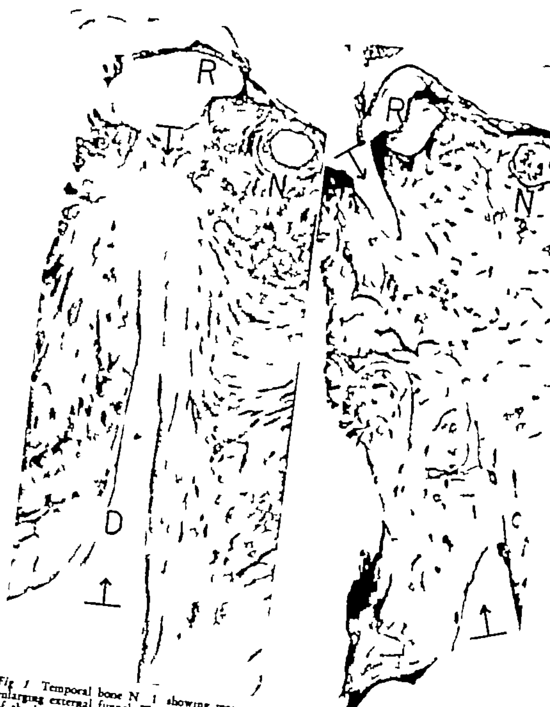
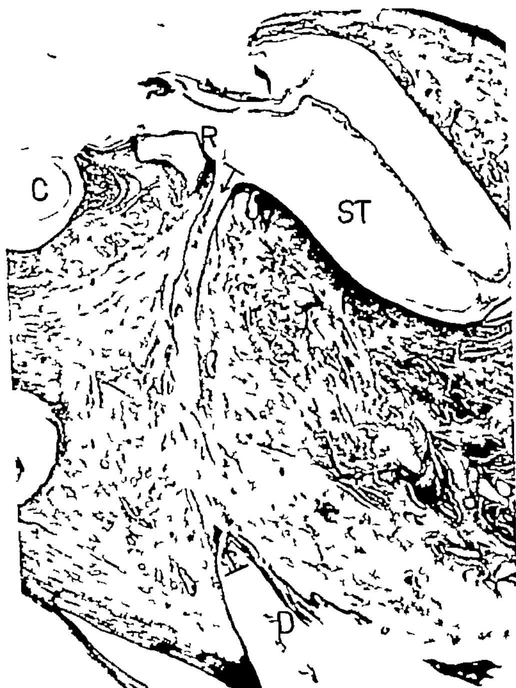


Fig 1 Temporal bone N 1 showing main part f the cochlea aqueduct with gradually enlarging external funnel. The length of the canal is 5.2 mm and its width 220 μ t the area of the bend. PAS stain magnif cation × 20 (left).

F g 2 Same case as in Fig. 1 section 200 μ deeper The opening of the aqueduct (width 380) adjacent to round window membrane is now clearly visibl R = round window membrane, N = round window branch f tympanic nerv D = dura. Arrows indicate the points between which aqueduct length w measured. Hemato lin-eosi stain, magnification × 20 (right).



F g 3 Temporal bone N 2, showing internal half of the cochlear aqueduct. The canal (between the arrows) is 5.8 mm long. The width f the queduct at its opening adjacent to the round window membrane (R) is 300 μ and 1 mm away from this point 310 μ. The aqueduct shows curved slope. V = inferior cochlear vein, ST = scala tympani, C = semicircular canal, D = dura. Hematoxylin-eosin stain, magnification × 17



Fig 4 Temporal bone N 4 showing the narrowest cochlear aqueduct in the whole series. The section runs along the wall of the aqueduct for 3 mm length from the cochlear orifice; the open part is 60 μ wide; it has bony spiculum in the right wall and is filled partly with loose arachnoid tissue. R = round window membrane, V = inferior cochlear vein. Hematoxylin-eosin stain, magnification × 32.

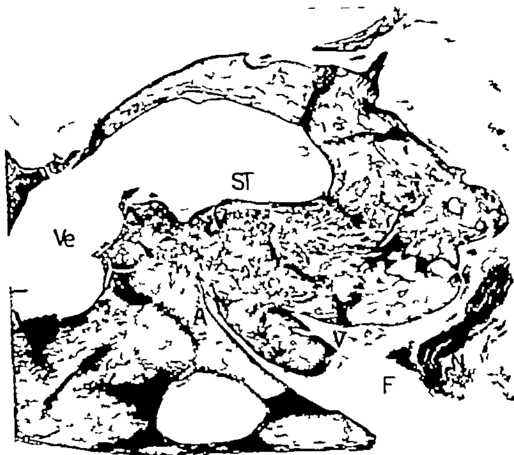


Fig. 5 Case 20, showing smoothly curved slope of the aqueduct (A) and a large funnel (F) containing the external part of inferior cochlear vein (V) and the glossopharyngeal nerve (N) with numerous corpora amylacea. The internal part of the vein (V) is seen adjacent to the depression of the internal orifice of the aqueduct. ST = scala tympani, V = vestibulum. Hematoxylin-eosin stain, magnification × 11.

The mean length obtained was 6.2 mm when including the funnel-shaped extension of the external orifice (no longer aqueduct proper) this adds an average extra length of 2 to 3 mm in various preparations.

The width of the cochlear aqueduct, measured from bone to bone, varied in considerable limits. The smallest diameter 80 μ was seen in case No 4 (Fig. 4), the only case in which the width of the canal was really narrow for a considerable length, 0.5 to 2 mm from the scala tympani. In all others, minimum width was at least 110 μ. The narrowest bone-to-bone distance occurred at an area 0.5 to 1 mm from the scala tympani and the canal rapidly widened at the internal orifice, the opening reaching a diameter between 210 to 600 μ (average 322 μ). Towards the external opening the width of the lumen increased steadily but more slowly. However at the funnel like common external orifice the recorded widths varied enormously from 690 to 2100 μ, depending upon the individual aqueduct and the course of the section.



Fig 6 Case 18 showing the internal orifice of the aqueduct (A) and an open lumen for distance of 1.5 mm. ST = scala tympani, Hematoxylin-eosin stain, magnification × 60 (left).

Fig 7 shows section of the same case 500 μ removed demonstrating oblique cross-sections of the aqueduct (A) and vein (V) due to an angled slope at the internal end. Hematoxylin-eosin stain, magnification × 63 (right)

It appears from the table that for cases 5 and 12, in which cross sections were employed, the maximal and minimal widths clearly indicate oval or elliptical shape of the canal This appears also from the diameter values for the external part of the aqueduct in cases 18 and 19 large values indicate that sections were cut obliquly along the long diameter



Fig. 8 Case 20 showing network of paracanals (P) starting from the external funnel (F) between the vein (V) and the external depression of the aqueduct (A). *ST* = scala tympani, C = semicircular canal, Hematoxylin-eosin stain, magnification × 13

The general slope of the aqueduct had two distinct forms. One was a slightly curved form (Figs. 3 and 5) without major deviations and the other a more or less straight canal (Figs. 1 2) combined in two (cases *18* and *19*) with a marked angle 1 mm from the scala tympani. There are examples of this latter type in Figs. 6 and 7 in which 1.5 mm of the aqueduct was seen in



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Fig. 10 Temporal bone No 7 showing large paracanal (P) from the middle of the aqueduct. The aqueduct (A) lumen is widely open while the lateral extension is filled mostly with dura periosteal tissue Hematoxylin-eosin stain, magnification × 15



Fig 11 Case 16, showing a short paracanal (P) parallel to the slope of the aqueduct (A), separated by thin bony septum. Both are lined by dura and rachnoid a d communicate with each other Hematoxylin-eosin stain, magnification × 165

parallel section but the course then changed at an angle of about 60—70 into cross sections continuing up to the external orifice.

While the bony walls of the cochlear aqueduct were in most cases smooth, some showed many irregularities This was the rule at the external orifice of the aqueduct where in addition to the lumen proper there were sometimes several channels starting from the funnel and extending for varying distances, 0.5 to even 5 mm into the bone outside the aqueduct (Fig. 8) Some of these canals had an unmistakable lumen lined by dural tissue which finally totally filled the blind end in the bone, while some of the side-channels — filled with loose connective tissue — led to the air cells of the temporal bone (Fig 9) In other cases, large side-canals opened from the mid-portion of



Fig. 12. Case 16, showing a 0.7 mm long paracanal (P) extending laterally from the aqueduct (A). Several corpora amylacea are seen in the aqueduct lumen. Hematoxylin-eosin stain, magnification × 130.

the cochlear aqueduct to end in blind pockets, filled with dura, in the temporal bone (Fig. 10). In several ears these paracanals were only 0.5 to 1.0 mm in length, running parallel (Fig. 11) or transversely (Fig. 12) to the aqueduct and only by studying the consecutive sections could a connection between the two be established. In several specimens bony spiculae (Fig. 13) and once an exostosis like formation (Fig. 14) projected from the canal wall, and the latter caused distinct narrowing of the aqueduct lumen. In no case, however was there even a suggestion of bony occlusion of the aqueduct.



Fig. 13 Temporal bone No. 10 showing the area of the external funnel with a prominent bony spiculum on the right side. Dura (D) is strongly adherent to the spiculum and between dura and arachnoid (A) and attached to the arachnoid, are some corpora amylacea. Hematoxylin-eosin stain, magnification X 70.



Fig. 14 Temporal bone No 4 with an exostous formation on the right wall. The canal lumen has width of 170 μ at area f the arrow and has loose arachnoid tissue and a few corpora amylacea in the lumen. Hematoxylin-eosin stain, magnification × 65



Fig 13 Temporal bone No 8, demonstrating a narrow (50 μ) paracanal (P) extending from the middle ear side f the round window membrane (R) into bone along the inferior cochlear vein (V) The paracanal I covered with middle ear epithelium and is filled with loose connective tissue PAS stain, magnification × 60.



Fig. 16 Temporal bone N 13 with 3 mm long and narrow (70 μ) paracanal (P) in the scala tympani (ST) starting 0.7 mm away from the insertion of the round window membrane (R). The paracanal is situated 200 μ deep of the cochlear aqueduct. Hematoxylin-eosin stain, magnification × 50.

In addition to side-channels starting from the external funnel or from the cochlear aqueduct, there were, close to the internal part of the aqueduct (or cochlear vein channel) parallel bony canals which started from the middle-ear or scala tympani side of the round window membrane (Figs. 15 and 16). Similarly as the channels starting from the external orifice, these canals filled with connective tissue, attained a length of 2 to 4 mm and ended blindly in bone.



Fig 17 Temporal bone No 6, showing external half of the aqueduct with the glossopharyngeal nerve (N) at the funnel. The aqueduct (A) measures 380 μ in width at the upper part of the visible lumen. Near the funnel the section approaches the wall and shows prominent dura. IM = internal acoustic meatus. Hematoxylin-eosin stain, magnification × 11

B SOFT STRUCTURES WITHIN THE AQUEDUCT

1 DURA AND ARACHNOID

During trimming of the bones before decalcification a considerable part of the soft structures in 12 temporal bones were sharply removed with a knife in the area of the funnel with a view to having a well identifiable external opening when cutting the sections. Therefore, only 6 specimens showed the glossopharyngeal nerve (Figs. 5 8 17) which crosses the area of the external funnel. However removal of any soft parts inside the channel was avoided and the dura and arachnoid were identified inside the channel lumen in all preparations. The dura was thin (from 5 to 20 μ) in the sections which were cut parallel to the canal (Figs. 1 3—6 13—14) in its midline, and in all transversal sections (Figs. 7 11—12) In some cases the dura was artificially separated from the bone, an extradural slit being formed. Some sections conveyed the impression that the dura was very thick and practically occluded the lumen. This, however was because the section at that point did not pass through the free middle part of the aqueduct lumen but along the dural cover sections at the midpoint in the same area always showed the lumen to be well open

The arachnoid lining (Figs. 4 11—14) of the aqueduct was demonstrable in most preparations as a very thin cellular layer sometimes disarranged, generally lying on the dura although in some slides the arachnoid was apparently torn and there was a subdural space between the dura and the arachnoid.



Fig. 18 Temporal bone No 2, showing several round, oval or longitudinal corpora amylacea in the aqueduct lumen which has a width of 300 μ. The corpora amylacea are partly free in the lumen, partly attached to arachnoid tissue Hematoxylin-eosin stain, magnification × 180.



Fig. 19. Case 17 showing the external funnel (F) with the widely open aqueduct (A) lumen and the external part of the inferior cochlear vei (V). Proteinous precipitate together with several corpora amylacea is seen adjacent to dura. Hematoxylin-eosin stain, magnification × 31

2. CORPORA AMYLACEA

Corpora amylacea were regularly seen inside the cochlear aqueduct. In some cases they were long measuring about 100 μ, but only 10 μ in thickness in some they were shorter and more or less elliptical or oval in shape (Figs. 12—14) In some areas the corpora amylacea were gathered into larger clusters and then presented a more rounded appearance with concentric nuclei in their central parts (Fig 18) Corpora amylacea were most frequent in the funnel area (Fig 19) and close to the funnel inside the aqueduct, but they appeared in smaller numbers along the arachnoid lining of the aqueduct up to the scala tympani (Figs. 20—22) In none of the cases was the impression

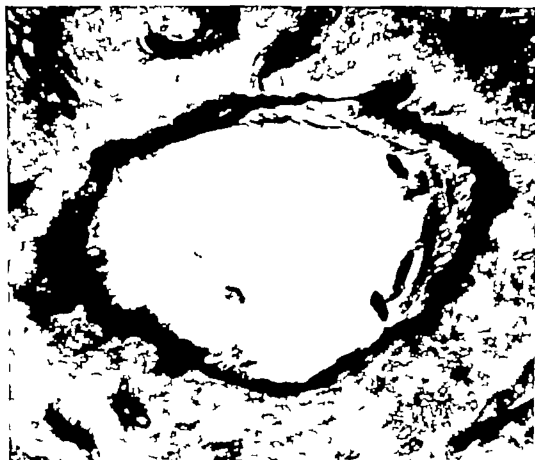


Fig 20 Temporal bone No 12, showin rounded transverse section of the aqueduct 250 μ away from the scala tympani. Horizontal width 550 μ, vertical 350 μ. Dura and arachnoid cover the walls, a few corpora amylacea adhere to the arachnoid in left wall. Hematoxylin-eosin stain, magnification × 180.

gained that the corpora amylacea filled the aqueduct lumen to the extent of restricting the fluid flow

Large numbers of corpora amylacea of varying sizes and developmental stages were seen in the frozen sections. They were most frequent in the boundary between the dura periosteum and the loose arachnoidal structures, and sometimes inside the glossopharyngeal nerve. The earliest stage was a whorl-like accumulation of degenerating arachnoidal cells and fibres accompanied by proteinous precipitates. After contraction and sequestration of these components, basophilic and weakly metachromatic clusters were left in the sections. In the next stage the material at their centre was contracted to a dense, hyalinized and strongly refractile core, whereas the outer borders remained basophilic.

The corpora amylacea thus seemed to develop as a result of degeneration and contraction (sequestration) of cells and fibres infiltrated by calcium salts and proteins. In cross sections the corpora amylacea had the appearance



Fig 21 Temporal bone No 5 showing an elliptical, oblique section of the aqueduct at the area of the bend 0.4 mm away from the scala tympani. Vertical width 230 μ. Dura and arachnoid follow the walls, some corpora amylacea are attached to the arachnoid. Hematoxylin-eosin stain, magnification × 125

of rounded grains well known as psammoma bodies in the pathology of meningeomas. In longitudinal sections the refractile core appeared laminated, resembling glistening yellowish canes. The basophilic wall seemed to contain rests of the cell nuclei.

In the initial cellular and fibrous aggregates there was nothing to remind one of former vascular structures, nor was the refractile central material reminiscent of cells.

As seen in figures 19—22, the corpora amylacea, in this temporal bone series, occurred either freely in the aqueduct lumen, partly attached to the arachnoid, or rarely below the arachnoid in the dura (Fig. 23). They were seen in abundance at all stages of formation at the external orifice where the end products were demonstrated also in the paracanals outside the aqueduct, as well as around the inferior cochlear vein during its course towards the inferior petrosal sinus. They were also frequently seen in the internal acoustic meatus around and in the cochlear nerve near the spiral ganglion but only in two temporal bones were such bodies seen in the lumen of the inferior cochlear vein (Figs. 24—25).



Fig. 22. Temporal bone No 2, showing internal orifice of the aqueduct. The lumen here measures 300 μ and contains many corpora amylacea surrounded by arachnoid tissue meshwork. The dura is thin and adherent to the walls. V = inferior cochlear vein, ST = scala tympani, R = round window membrane. Hematoxylin-eosin stain, magnification × 70.



Fig. 23 Temporal bone N 10, showing bony spiculum with several *corpora* myl inside the dural tissue Hematoxylin-eosin stain, magnification × 180



Fig 24 Case 16 showing a large corpus amylaceum inside the inferior cochlear vein (V). Another corpus amylaceum is seen at the orifice of the cochlear aqueduct (A). Hematoxylin eosin stain, magnification × 38



Fig. 25 Case 16, showing the collecting vein near the external funnel, (before the inferior cochlear vein joins it) with several *corpora amylacea* inside. Haematoxylin-eosin stain, magnification × 200.



Fig. 28 Temporal bone N 15 showing an oblique view of the inferior cochlear vein 2 mm away from the internal orifice. The thin endothelium is surrounded by loose connective tissue meshwork. Hematoxylin-eosin stain, magnification × 150.

The vein wall is made up of very thin endothelium (Fig 24), which, supported by the bony walls of the channel, clearly differs from the thick wall of ordinary veins. A fine meshwork of connective tissue supports the thin vein wall in the bony canal (Fig. 28) The vein channel then continues parallel with the bony cochlear aqueducts towards the external orifice the distance as a rule grew larger being on average 700—900 μ removed from the aqueduct. In 3 cases the distance exceeded 1 mm near the external funnel (Fig. 29)

Measurements of the width of the vein channel were also made in 18 temporal bones. At the floor of scala tympani the vein averaged 150 μ, while during its proper course, 0.5 to 1 mm from the scala tympani, it was clearly larger than the aqueduct, measuring about 150—200 μ. During its further course the individual and local differences in vein width were greater than those for the aqueduct, but no great average difference between the two was observed. In cross sections, the vein had a more roundish form than the aqueduct.

The inferior cochlear vein in two cases joined another somewhat larger transversal vein near the external funnel (Figs. 30—31) the origin of this vein could not be determined. In other specimens the vein had a straight course into the external funnel where it started a slope anteromedially at an angle of about 60—70 and had a course towards the inferior petrosal sinus, although the junction was not included in the specimens.



Fig 29 Case 17 showing the slightly curved slope of the inferior cochlear vein canal (V) from the scala tympani (ST) to the external funnel (F). The latter contains the glossopharyngeal nerve (N) and the whole base reveals many corpora amylacea in various developmental phases. Hematoxylin-eosin stain, magnification × 11



Fig. 30—31 Case 16, showing joining f the inferior cochlear vein (V) with a larger transversal ein canal. The sections are 150 μ part. Hematoxylin-eosin stain, magnification × 40.

4 THE BARRIER MEMBRANE

Considerable attention was directed to the medial end of the cochlear aqueduct at the scala tympani. In this material a barrier membrane, similar to that presented by Waltner was clearly seen in one specimen belonging to the series of perilymph albumin analyses and in one case of the 5 temporal bones with an intact round window membrane (Figs. 32 and 33) The covering endothelium seems to be a direct continuation of the lining of the scala tympani endothelium crossing over the cochlear aqueduct to join in the round window membrane. It consists partly of a thin layer of cells and partly of basophilic arachnoid mesh type connective tissue. In all other cases there was no evidence of any membrane closing the orifice of the cochlear aqueduct (Figs. 2—3 6 22)

On the other hand, the nutrition canals starting from both sides of the round window membrane and extending deep into the bone, running parallel to the cochlear aqueduct, all had a barrier membrane» covering their orifice (Figs. 15 and 16) This membrane was a direct continuation of the endothelial lining of the neighbouring bone



Fig 32. Temporal bone N 14 showing the orifice of the cochlear aqueduct t the scala tympani (ST) The endothelium continues as kind of barrier membrane (B) over the orif ce to the insertion f the round window membra e. Cochlear aqueduct (A) lumen containing arachnoid-type mesh under the membrane with wide lumen. I the lower part of the lumen, separated in the area of the bend by bone, there are dura, arachnoid and some corpora mylacea to be seen. Hematoxylin-eosin stain, magnification × 80



Fig. 33 Case 16 showing »barrier membrane» (B) consisting of endothelial cells and arachnoid-type mesh with basophilic staining. The cochlear aqueduct (A) is wide open. Hematoxylin-eosin stain, magnification × 525.

C. ALBUMIN MOBILITY OF PERILYMPH AND CSF CORRELATED WITH COCHLEAR AQUEDUCT PATENCY

Of the 12 specimens (Table II) included in the perilymph mobility studies (Palva and Raunio, 1968) 6 showed eosin dye in the scala tympani orifice of the aqueduct following injection of dye into the external funnel. In three of these cases (1 3 and 15) the mobility of perilymph and CSF albumin was identical and faster than that of serum albumin. In cases 12 and 14 serum albumin had the lowest mobility CSF albumin was somewhat faster and perilymph showed the fastest moving albumin In case 13 serum and CSF albumins had identical mobility but perilymph albumin was clearly faster

The remaining 6 cases, with negative injection of eosin included cases 2, 9 *and 10 in which perilymph and CSF albumin had identical mobility both* being faster than serum albumin Whereas one case (5) showed identical mobility for all three fluids, the mobility of serum and perilymph albumin in another case (6) was similar and slightly faster than that of CSF albumin. In the third case (7) the fastest albumin was recorded in perilymph while the mobility of CSF was somewhat slower than that of serum albumin.

TABLE II

Albumin mobility and cochlear aqueduct patency

Case	Albumin Mobility			Eosin Injection	Minimum Width of Aqueduct (μ)
	Serum	CSF	Perilymph		
1	2.022	2.073	2.065	+	150
2	2.029	2.074	2.064		180
3	2.086	2.205	2.190	+	150
5	2.205	2.195	2.189		150
6	2.118	2.091	2.114		150
7	2.164	2.143	2.188		140
9	2.137	2.176	2.180		150
10	2.075	2.134	2.146		150
12	2.072	2.095	2.146	+	150
13	2.092	2.094	2.148	+	120
14	2.085	2.108	2.118	+	120
15	2.081	2.141	2.137	+	130

V DISCUSSION

Despite a number of thorough and painstaking studies on the anatomic variations in the cochlear aqueduct in the early part of this century several questions have remained open. Owing to the fact that the aqueduct has not been routinely sectioned parallel to its slope, measurements of its length and width and data on its patency have apparently been less accurate

The length of the canal has varied widely from one report to another Most of the earlier studies stated that the average length of the bony aqueduct exceeded 10 mm being generally 10 to 15 mm. Indeed Meurman pointed out that in adults the length of the canal is rarely less than 10 mm.

Our figures for the length of the aqueduct (Table I) showed some variation from case to case (range 5 to 8 mm) with an average of 6.2 mm. The only study in which the length of the aqueduct is similar to our measurements is that of Ritter and Lawrence who arrived at these figures (average 6 4 mm) by direct dissection of the aqueduct. Since our data are mainly based on sections parallel to the canal, the measurements can be made accurately and we feel confident that these figures, as well as those of Ritter and Lawrence, represent the real length of the aqueduct. However the external orifice funnel was not included in these measurements and this apparently is one of the reasons why the above figures are smaller than previous ones. Examination of for example, Meurman s table II giving the width of the aqueduct, clearly shows that at least part of the external funnel was included in the length calculations.

If the cochlear aqueduct was short, 5 to 6 mm, it generally had a straight slope except near (0.5 — 1.0 mm) the internal orifice at the scala tympani. Here there was often a slight bend of the order of 200 μ and the whole canal could not be shown in one section. In two cases (18 and 19) the bend approached 110—120 and, after demonstration of the straight course during the last 1 mm the following parallel sections showed oblique cross sections (Fig 7) In cases with a longer canal, the slope was generally slightly curved and sometimes a considerable angle formed between the two ends, at the funnel and in the scala tympani (Figs. 3 and 5)

As regards the width of the aqueduct, our results differ considerably from those of Ritter and Lawrence. Their finding of an hour-glass aqueduct, most narrow at the middle area (average 90 μ) is not corroborated our results substantiate the findings made by the early investigators that the narrowest point is near the cochlear orifice. This may vary slightly from case to case but is generally 0.5 to 1.5 mm away from the scala tympani. On the other hand, their observations of an elliptical form of the cross section was corroborated with the long axis of the oval transverse to the long axis of the petrosa.

Our canal width measurements (Table I) agree in principle with Meurman's data although our minimum figures are larger The general increase in canal width from the point of about 1 mm from the scala tympani towards the external funnel also essentially accords with Meurman's measurements but our rate of increase inside the aqueduct proper is somewhat less. This probably is due to the fact that some of Meurman's figures refer to areas outside the aqueduct proper

The paracanals demonstrated by us in several specimens were discussed briefly by Karlefors, who found that fluid from the scala tympani could be forced into the CSF space by routes other than the cochlear aqueduct. The bony canals starting from either side of the round window membrane run for various lengths parallel to the region of the aqueduct although in our study they were not seen to merge with it these apparently are partly normal nutrition canals of bone and may partly represent attempts at duplication of the aqueduct. Some of the side channels clearly connected the cochlear aqueduct with the neighbouring bony air cells or Volkman's canals. Several shorter or longer channels started particularly at the external orifice of the cochlear aqueduct and then ran parallel to the aqueduct from the funnel into the bone, sometimes close to the scala tympani. In all cases, even if the width of the paracanals approached that of the aqueduct proper they were found to end blindly in bone.

A connective tissue similar in structure to dura generally filled the paracanals whether they started from the external funnel or from the aqueduct itself If serial sections had not been studied, it would have been possible in some cases to consider the paracanals wrongly as the aqueduct proper This might have led to the conclusion that the aqueduct was first blocked with connective tissue and finally occluded totally by bone. The paracanals starting centrally had a lumen containing a loose connective tissue meshwork.

In some specimens bony spiculae appeared along the course of the aqueduct but the external orifice showed irregular bony contours in all ears. In one temporal bone (Fig. 14) the aqueduct wall showed formations suggesting the endosteal bone proliferation described by Meurman, to which he also had attributed total closure of the canal in advanced age. Even though the canal in this case of ours was the narrowest (80 μ) the lumen remained patent for the whole course We must also take exception to Meurman's opinion that bony changes are caused by old age since 5 of our 14 cases were over 70

Our findings differ basically from Meurman's conclusions in that we could not demonstrate any bony occlusion of the aqueduct, nor did we find even indications of total soft tissue obliteration. Whereas Meurman claimed this latter to be the rule, we demonstrated that all canals in the present series had an open lumen. The free space in the cochlear aqueduct always clearly exceeded the space occupied by the soft structures on the walls, which at the midpoint varied between 5 to 20 μ. Since it was the handling of the specimens that apparently caused separations of the arachnoid tube from the

dura and also of the dura from the bony walls there is no reason during life — when both the dura and arachnoid are near the bony walls — to doubt the patency of the lumen

The idea that soft structures occlude the aqueduct in our view derives from the fact that, in the areas where there are bends in the aqueduct tangential sections may show no lumen at all the dura and arachnoid taking up the total space. We suspect that the soft tissue obstructions observed by Meurman exactly where the bone-to-bone distance is the shortest and where the aqueduct turns before entering the scala tympani are due to this section effect and thus do not represent real soft tissue obstructions. Another reason may be that the long para aqueducts ending only a short distance from the floor of scala tympani, may have been taken for true aqueducts.

As pointed out by Waltner the corpora amylacea in this area can be considered specific to the cochlear aqueduct and we have only in two specimens found them inside the inferior cochlear vein. Our observations as regards shape and size of these bodies concur largely with those of Meurman and of Waltner However we cannot agree with Waltner in considering that the function of the corpora amylacea is to control and prevent the free passage of fluid into the scala tympani Their presence at various places at the skull base argues against this specific functional hypothesis and their presence suggests only a perilymph richer in protein than in several other areas of the CSF-system. Our detailed studies of the corpora amylacea in frozen sections indicated that they originate from degenerating arachnoid cells and fibres encircled by proteinous precipitate and infiltrated by calcium salts.

A barrier membrane at the orifice of the cochlear aqueduct in the scala tympani was found in 2 of the 20 temporal bones. The membrane seemed to effectively obstruct the passing of erythrocytes from the scala tympani into the aqueduct. However since the membrane consisted of only normal endothelial cells and arachnoid-type tissue it probably has no specific ability for selective filtration comparable to the specialized cells of Reissner's membrane.

In the majority of the specimens studied, there was not the slightest sign of such a membrane and it must be concluded that as a rule the fluid exchange between the CSF space and the scala tympani is not obstructed by any membrane The same conclusion was also reached later by Altmann and Waltner (1947) in their studies on rabbits, in which a continuos membrane could not be demonstrated in all consecutive sections.

The patency of the aqueduct might be questioned, in spite of all the above evidence, by pointing to our eosin injection experiments in which only 6 out of 15 temporal bones apparently had an open lumen. This, however was due to a technical fault: figures 5,8 19 and 29 show convincingly how easy it is to insert the tip of the needle wrongly i.e so that the proper site of injection at the external opening of the aqueduct, is missed.

The inferior cochlear vein contains a separate channel, parallel to the-

cochlear aqueduct. In addition to the very thin endothelial wall of the vein, the channel showed loose connective tissue. The width from bone to bone was approximately the same as that of the cochlear aqueduct — with occasional larger lacunae — but in cross-sections the vein had a more rounded diameter than the elliptical aqueduct. At the scala tympani, the vein could always be distinguished from the aqueduct because of its location further off from the round window membrane the distance between the vein and aqueduct walls averaging 300 μ.

During their course in the temporal bone the two structures could be distinguished because the cochlear aqueduct contained both dura, arachnoid and corpora amylacea, which all were absent from the cochlear vein channel, apart from corpora amylacea in two cases. In none of the specimens did the vein run in the channel of the cochlear aqueduct and the distances between the two increased towards to external funnel (average 800 μ). Thus, the main observations of Karlefors and Meurman were confirmed.

This study was started because we found that the mobility of perilymph albumin corresponded on average to that of CSF albumin and both of these had a faster mobility than serum albumin. This suggested that perilymph albumin was derived from the CSF space, which was further supported by the appearance in the perilymph of a fast prealbumin fraction (Palva and Raunio 1967 a and b), identical in disc electrophoresis to that of brain tissue. Obviously it seemed, there must generally be a functioning connection between the perilymph and the CSF space, though most earlier reports on the cochlear aqueduct testified to the contrary. The cochlear aqueduct, however need not be the only pathway for passage of fluids the vestibular aqueduct might serve as a connecting link between the CSF and perilymph in the vestibule, and the spinal fluid may have access into the inner ear along the foramina of the VIII nerve fibres. Nevertheless, since the cochlear aqueduct is the logical pathway for any major exchange of fluids, the special aim of the study was to examine whether this pathway was patent or not.

The twelve temporal bones subjected to mobility studies included 6 in which the CSF and perilymph had similar and greater mobility than serum albumin three of these ears had a cochlear aqueduct widely open to eosin injection with slight pressure. There were 3 additional cases in which the perilymph showed higher albumin mobility than the other two fluids and 3 cases in which perilymph and serum albumins had identical mobility.

In all temporal bones studied in this series, the cochlear aqueduct was anatomically patent though it varied in width. Thus there can be no doubt as to the possible pathway for exchange of fluids between the CSF space and the perilymph. The fact that albumin mobility studies do not in all cases indicate a similar mobility of perilymph and CSF albumin seems partly due to the contamination of fluids from the small blood vessels and capillaries always present in post mortem temporal bones. This has become apparent particularly in researches for determination of the ionic concentration of

human labyrinthine fluids (Rodgers and Chou 1967 Palva and Tikanmäki 1968 Palva and Raunio 1968)

Considering that in most post mortem specimens the average values for CSF and perilymph albumin mobility are identical and faster than for serum albumin, this strongly favours the view that perilymph albumin really originates from the CSF space. If it did derive solely from the blood, viz. from the capillary network in the walls of the perilymphatic space, then the post mortem analyses should show no difference in the mobility of these two albumins. The slightly faster mobility of CSF albumin compared with serum albumin has been demonstrated in immunoelectrophoretic studies (Lowenthal et al., 1960)

Our observations on the patency of the cochlear aqueduct correlated very closely with the pertinent clinical reports by Perlman and Lindsay Crowe, and Schuknecht et al. The apparently obvious difference between children and adults as regards patency of the cochlear aqueduct in purulent meningitis, seems to be explained by the fact, already mentioned by Crowe, that in adults the longer cochlear aqueduct with its funnel both containing soft tissue in abundance can be temporarily closed by inflammatory swelling of the arachnoid and dura. Thus, in adults a purulent infection seldom reaches the inner ear though a non-inflammatory disease, such as subarachnoid hemorrhage, can result in an abundance of erythrocytes in the scala tympani. In children, in whom the cochlear aqueduct including its external funnel is shorter than in adults, the infection may more easily penetrate into the cochlea. In some ears, the barrier membrane, when it occasionally exists, can also prevent the spread of bacterial infections into the inner ear This, however must apply to both children and adults since there is no reason to suppose that the membrane would develop only later in life

Naturally our study does not explain the role of the cochlear aqueduct in labyrinth physiology as such, but it supplies a firmer basis for speculations. We have no doubt that part of the perilymph derives from the capillary network in the cochlea but think that the majority of the fluid, particularly during life derives from the CSF space. It is equally obvious that these two are not the sole sources of various substances in the perilymph some of the more complex substances, particularly enzymes, apparently originate from the sensory cells of the organ of Corti. These cells have a lively metabolism and being surrounded by fluid which is in connection with perilymph, these constituents of the cellular metabolism have access to the perilymph.

The animal experiments of Schuknecht and Seifi (1963) showed no evidence of operative closure of the cochlear aqueduct resulting in altered conditions in the cochlea When the endolymphatic sac or duct was blocked (Kimura and Schuknecht 1965), a distension of the dolymphatic space was demonstrated later at the time the animals were killed. Thus the cochlear aqueduct as such may not be necessary for the maintenance of fluids in the inner ear

An interesting personal observation was made some years ago by Arny g

(1963) who had a lumbar puncture made on himself this was followed by symptoms resembling Ménière's disease for a short time The symptoms were thought to arise from a decreased fluid pressure in the subarachnoid space possibly causing a temporary reversal of the general direction of the fluid flow from the CSF space into the scala tympani.

In Ménière's disease particularly more information on the dimensions of the cochlear aqueduct is required. Hitherto attention has been mainly focussed on endolymphatic hydrops. Data on aqueduct width and patency in confirmed cases might add valuable matter for speculation on the mechanism of this disease

VI SUMMARY

Previous studies on the human cochlear aqueduct were reviewed, paying special attention to the early work of Karlefors, Meurman and Karbowski. Analysis of their results and more recent data showed that the patency of the aqueduct, in particular caused division of opinion several other structural characteristics also seemed insufficiently known.

The material consisted of 20 temporal bones 12 of these were removed for comparison of serum perilymph and CSF albumin mobility. In 15 ears perilymph had been removed through the round window membrane but in 5 the scala tympani had not been opened. The bones were serially cut to 25 μ thickness. In 18 cases the sections were made parallel to the aqueduct and each consecutive section was examined.

The length of the canal averaged 6.2 mm when measured from the orifice at the scala tympani to the bottom of the external funnel. This agrees with the results of Ritter and Lawrence and it is concluded that the length reported in most earlier studies is not realistic for the aqueduct proper. If the walls of the external funnel are included, a somewhat bigger length is obtained, but even then the average length will not exceed 10 mm.

The slope of the aqueduct, when short, was nearly straight, but several longer canals presented a considerable curve in their slope from the external funnel to the internal orifice. Five specimens showed a marked bend from a straight base line during the last 1—1.5 mm from the internal orifice (30—40° in three specimens and 60—70 in two). These irregularities caused failure of the demonstration of the whole aqueduct in one section study of consecutive sections, however always gave a clear picture of the aqueduct dimensions.

The smallest width of the aqueduct was recorded 0.5 to 1.5 mm from the internal orifice, and minimum width varied from 80 to 150 μ. In both directions width increased consistently — most abruptly at the orifice of the aqueduct at the scala tympani, although at the external funnel the lumen was two to four times as large as at the internal orifice. The aqueduct lumen was oval or elliptical. Although it was generally smooth-walled, there were specimens in which bony spiculae extended from one wall into the canal parallel to its lumen once an exostosis like formation narrowed the lumen considerably. There were no bony obliterations.

The aqueduct lumina contained extensions of the dura and arachnoid up to the internal orifice, where the lining was smoothly transformed into the endosteal covering of the scala tympani. Even if in some of the parallel sections traversing the margin of the canal the dura appeared thick and seemed to occlude the canal, in sections through the midline the lumen was always

patent and the soft tissues were no more than 5 to 20 μ in thickness. There was no evidence to support the earlier claim that a soft tissue obliteration of the canal was the rule

There were several paracanals starting from the external funnel and varying in depth from 0.5 to 4 mm these terminated blindly in dura like tissue or opened into bone spaces containing air cells. Similar but generally much shorter extensions started from the aqueduct proper Paracanals around the internal orifice started close to the round window membrane insertion on either side and sometimes extended as deep as 3 to 4 mm at a distance of only 100 μ from the aqueduct or the inferior cochlear vein These internal orifices always terminated in the loose endosteal connective tissue, covered by a thin endothelium. They were thought to have a function comparable to normal nutrition canals of bone, while some of those starting from the external funnel might represent attempts at duplication of the canal.

Corpora amylacea were constantly present, particularly in the whole external funnel comprising the area of the inferior cochlear vein and the glossopharyngeal nerve, and in the aqueduct proper up to the scala tympani. With iodine these bodies stained weakly in fresh frozen soft tissue preparations removed from the funnel Various staining techniques showed that the majority of the corpora amylacea develop as a result of degeneration and contraction of arachnoid cells and fibres infiltrated by calcium salts and proteins precipitates In two temporal bones, corpora amylacea were seen also inside the inferior cochlear vein and more regularly in the internal acoustic meatus. Although there were many corpora amylacea in some sections, there was nothing to suggest that they might occlude the fluid exchange between the perilymph and CSF

In two temporal bones there was a barrier membrane at the mouth of the internal orifice as demonstrated by Waltner This membrane seemed to consist of a arachnoid-type tissue network rather than a real endothelial membrane. In all other specimens the aqueduct lining continued towards both the round window membrane and the apex of the cochlea, joining the thin endothelial lining of the scala tympani. The interchange of perilymph and CSF was thus not restricted by any obstacle at the internal orifice of the aqueduct in 90 % of the specimens It was felt that even the membranes did not much hinder the fluid exchange although it might restrict the passing of larger corpuscles.

The inferior cochlear vein was demonstrable in 18 of the specimens starting as a boomerang like curved structure about 300 μ towards the apex from the internal orifice of the aqueduct the latter was adjacent to the insertion of the round window membrane The vein channel diameter was roundish but otherwise the dimensions did not differ appreciably from those of the aqueduct The distance between the two varied and increased towards the external funnel up to a maximum figure of 1.2 mm at the external funnel the vein made a 60—70 turn towards the inferior petrosal sinus The vein channel had a thin endothelial vein wall surrounded by loose connective tissue.

This study was initially stimulated by the finding that perilymph albumin

mobility on average equalled that of CSF and both were faster than serum albumin. Immunologically perilymph albumin seems to be similar to CSF albumin and it is reasonable to assume the latter to be the source of the former. This finding was further substantiated by the fact that perilymph contained fast-moving prealbumins identical to those of CSF and brain tissue extract, but lacking in serum. The present studies on cochlear aqueduct patency definitely demonstrated that this type of fluid exchange through the cochlear aqueduct is fully feasible. The other possible pathways, viz. the vestibular aqueduct and the foramina of the VIII'th nerve fibres, may play a minor role.

That the cochlear aqueduct in adults seldom transmits purulent infections into the inner ear despite its patency to erythrocytes was attributed to the fact, already pointed out by Crowe, that the soft tissues of the combined funnel and aqueduct, particularly narrow 0.5 to 1.5 mm from the internal orifice, are capable because of infectious swelling to hinder the transmission of bacteria into the inner ear. In 10 % a membrane across the aqueduct mouth at the scala tympani might be of further assistance.

Finally it is felt that the course and structural status of the cochlear aqueduct is insufficiently known for instance in cases of Ménière's disease. Further studies based on temporal bones of autopsied patients might shed additional light on the problematic issues of this disease.

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HEARING IMPAIRMENT IN TURNER'S SYNDROME

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SUPPLEMENTUM 247

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CONTENTS

SUMMARY

The hearing was studied in 79 clinically and cytogenetically analysed patients with Turner's syndrome of different ages. Middle ear infections had occurred in 52 out of 76 patients (68 per cent) generally during childhood. Six of the patients had chronic infections after the puberal years. Accordingly 17 patients (22 per cent) showed a conductive or mixed type of hearing impairment. Cephalometric analysis showed the external auditory meati to be caudally displaced which points towards an abnormal orientation of the Eustachian tube, a condition that may explain the predisposition to middle-ear infections. Other infections were apparently not overrepresented. Sensory neural hearing impairment with recruitment was found in 51 patients (64 per cent) generally as a bilaterally symmetrical dip in the audiogram, generally centered around 2 kHz (42 per cent). This finding is interpreted as a defect in the outer hair cells of the organ of Corti localized to the upper part of the basal and the lower part of the middle coils of the cochlea. Only one of the 10 children demonstrated hearing impairment indicating a degeneration rather than a congenital malformation. There was however no striking progression in the later age groups. The aetiological significance of the abnormal sex chromosome constitution for the hearing impairment is discussed.

INTRODUCTION

The genetics of sensory-neural hearing impairment in man has not yet been clarified even if the recent application of more advanced audiometric test methods has shed some new light on this problem (Anderson & Wedenberg, 1968 a). Small but distinct dips in the middle frequency range of the hearing threshold and/or abnormally high thresholds for the acoustically elicited stapedius reflex could be demonstrated in an unusually high frequency among the carriers of a presumptively recessive gene. Among different types of hearing impairment dips are also found in a high proportion of supposedly homozygous propositi. A similar dip is commonly observed in patients with Turner's syndrome (short stature, ovarian dysgenesis and different malformations in females) (Lindsten, 1963). This is of special interest since Turner's syndrome is almost exclusively caused by welldefined numerical and structural abnormalities of the X chromosome (review in e.g. Lindsten & Fraccaro, 1965; Turpin & Lejeune 1965; Bartalos

& Baramki, 1967) It was therefore considered of importance to investigate in some greater detail the nature of the hearing impairment in these patients.

The present study which deals with this problem consists of three parts an interview regarding the previous history of middle ear infections, an audiometric analysis, and cephalometric measurements with regard to the position of the external auditory meati. In addition, tomography of the cochlea, analysis of the vestibular function and electroencephalogram were studied in parts of the case material.

MATERIAL

Seventy nine clinically and cytogenetically analysed patients with Turner s syndrome were used for the present study Their chromosome constitution and age at the time of the hearing investigation are given in Appendix 1 The eight infants were studied after their diagnosis had been made at birth because of the finding of oedema on the hands and feet, and loose skin folds in the back of the neck. The 71 non infants were studied when they sought medical advice because of short stature and/or primary amenorrhea

Forty five of the 71 non infants were randomly selected for the cephalometric analysis, which also included two control materials One consisted of 33 apparently healthy 11 years old girls corresponding to the case material with regard to body height. The other comprised 46 females, 16–28 years of age (mean age 20.5 years) with approximately the same age distribution as the patients (mean age 22.5 years)

METHODS

The previous *history of ear infections* was based upon an interview with the patient and/or her parents, and if possible on earlier hospital records.

Different *audiometric methods* were used to study the hearing The hearing threshold was determined by air and bone conduction octave audiometry on the following test frequencies 0 125 0.250 0.5 1 0 1.5 2 0 3 0 4.0 6 0 and 8 0 kHz. In order to obtain a more detailed picture of the hearing threshold continuous frequency Békésy-audiometry was used in many cases. Since this latter method gives an exact pattern of the hearing threshold for all frequencies it is possible to detect minor impairments which may not show up in the octave audiogram.

Acoustic recording of the intra-aural muscular activity (the stapedius

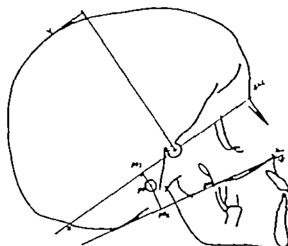


Fig. 1 Lateral projection. Cephal metric reference poi ts nd lines. The junction of line through nd *s*, nd the xternal surface of the occipital bone *n* the most ant rior point f the naso-frontal suture; *pm* poi t representing the dorsal margi of the maxill t the level of th nasal floo *po* the centre of the metal ring of the plastic rod f th cephalost t ; po_1 the perpendicula f *po* on *SNL*; po_2 the perpendicul f *po* on *NL*; *s* the ce ter of sella t rcica *p* the pe of the terior nasal spina; the uppermost poi t of the crani l va lt; *NL* line through *p* nd *pm*; *SNL* line through and *s*.

reflex) was used to distinguish between sensory neural and conductive hearing impairment, *i.e.* defects in the neural or the sound transmitting mechanisms, respectively This is not always possible with the ordinary audiometric technique It has earlier been shown that the presence of a stapedius response excludes any conductive component of clinical significance (Klockhoff 1961)

The stapedius reflex was also used to decide whether a sensory neural hearing impairment was of recruiting or non recruiting type According to Metz (1952) the impairment is connected with recruitment if the span between the elevated air conduction threshold and the threshold for the stapedius reflex is reduced.

Children too young to cooperate in the ordinary tone audiometric test were examined by play audiometry according to Barr (1955) Special methods were used to test the hearing of the children who were too young to cooperate also in play audiometry In these cases the auro-palpebral reflex (i.e. a contraction of orbicularis oculi muscle elicited by sound stimulation) and the awakening of the child by sound stimuli (Wedenberg, 1956 and 1963) were used. These methods make it possible to decide with reasonable certainty already from birth whether a child has a hearing impairment and also to some extent the type and degree of this impairment (Wedenberg, 1963)

The *cephalometric measurements* were performed on X-ray films taken

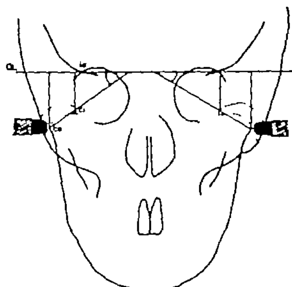


FIG. 2. Frontal projection. *Cephalometric reference points and lines. el* The midpoint of the ear drum; co: th junctl n of the lateral surface f th ramu of the mandible and the lower margi of the external audit ry canal; *l* : the junctio of the lateral border of the orbit and the gre t(er) wi g of the sphenoid bone; *OL* a line through *lo* both ides.

In a cephalostate In this device the head was fixed in the external auditory meati by plastic rods with metal indicators, a ring in the periphery of the left one and a central point in the right The median plane of the head was parallel with the film and the beam from the X-ray tube was centered on the rods The projection of the ring was used for localization of the external auditory meatus on the film The distances between the focus of the X ray tube on one hand, and the median plane of the head and the film on the other were kept constant

The following measurements were used to determine the position of the external auditory meatus In horizontal direction the distances (*s n*) (*n po*) (*s* po_1) and (*e* po_1) in vertical direction (*o-s*) (*po*-po_1) and (*po*-po_2) (for explanation see Fig 1) All measurements were transformed into indices because of differences in size of the skull between the patients and the control materials (Filipsson, Lindsten & Almqvist 1965)

In order to determine the slope of the external auditory canals 11 patients with Turner s syndrom and 11 control females of the same age were randomly selected and investigated as follows. The canals were inspected in order to exclude damage of the ear drum, cleaned from cerumen and filled with contrast medium (Pharmbaryt[1]) diluted in water to a consistency which made it suitable to exclude air close to the walls and the ear drum The meati were plugged with the plastic rods of the cephalostate described above and one X ray film was taken in frontal projection. The length of the auditory canals was estimated by projecting the points co and *el* on the

OL-line the slope was measured as the angle between a line through *co* and *ci* and the *OL*-line (Fig. 2)

Tests for significance were performed with Student s *t* test. A difference was considered statistically significant at the 0 01 level

The analysis of the vestibular function included registration of spontaneous and positional nystagmus and caloric and rotatory tests. The caloric test was performed according to Fitzgerald & Hallpike (1942) using water of 30 and 44 C temperature. The rotatory test included four different speeds 1 2, 4 and 8 /sec² It was started with acceleration to 40 80 120 and 160 followed by constant rotation for three minutes and then decelera tion Lag periods, duration of steady state and postacceleratory nystagmus were recorded.

RESULTS

MIDDLE EAR INFECTIONS

The detailed results regarding the previous history of middle ear infec tions are given in Appendix 1 Information could not be obtained for three of the patients.

Altogether 52 out of 76 patients (68 per cent) had had middle ear infec tions for which they had been treated by a physician. Thirty five out of the 52 patients had had one or a few such infections leading to spontaneous perforations of the ear drum or to surgical treatment (paracenthesis) The remaining 17 had had repeated infections for which they had been hospitali zed, and in four of these (nos. 10 34 39 72) middle ear surgery had been performed.

Regarding the role of ear infections as a possible cause of hearing im pairment, the following observations were made on the 76 informative patients. Twelve out of 26 patients with a normal hearing had suffered from ear infections. Ear infections were recorded for all 17 patients with a con ductive or mixed type of hearing impairment, and also for 24 out of 34 with the pure perceptive type of impairment The cell system of the mastoid processes was studied on X ray films and considered smaller than normal in five (nos. 20 29 54 56, 70) and normal in seven cases (nos. 14 15 25 26, 33, 46 73) Of these two and four patients, respectively had had ear infections.

The ear infections generally occurred bef re the age of 10 years, but six patients had chronic infections after the age when puberty normally occurs. Three of the eight patients below three ye rs of age had already had middle ear infections. Thus Turner patient seem t g t such infections to a rather high extent There is no uitable control material available however In our experience other types of infection are n t rrepresented among these patients.

Table 1 *Number of patients with Turner's syndrome classified according to age at the first examination and type of hearing impairment*

Age group (years)	Number of patients			Number of patients classified according to type of hearing impairment				
	Total	With normal hearing	With hearing impairment	Mono- and bilateral dips	Monolateral dip, mixed other side	Other sensory neural types	Bilaterally mixed	Conductive
0–9	10	9	1	1	—	—	—	—
10–14	5	—	5	2	2	1	—	—
15–19	32	9	23	11	1	2	1	5
20–24	17	5	12	9	1	2	—	—
25–29	6	1	5	—	1	2	2	—
> 30	9	2	7	4	1	2	—	—
Total	79	26	53	27[b]	6	9	3	5

This patient had monolateral dip and pure conductive impairment on the other side
[b] Five patients had a monolateral dip, the others bilateral. One patient with monolateral dip had flat loss on the other side.

AUDIOMETRIC ANALYSIS

Definitions (see also Appendix 1) The *hearing* was considered to be within *normal* limits if the hearing threshold was below 20 dB in the frequency range 0.125–8.0 kHz. From this follows that the *hearing* was considered to be *impaired* if the threshold was at least 20 dB or more, and if the impairment covered at least one octave *Recruitment* was defined as the phenomenon which causes the sensation of a tone to rise more rapidly with increasing sound intensity than in the case of the normal ear Hearing impairments with recruitment are considered to be caused by changes within the cochlea (Dix, Hallpike & Hood, 1948)

Hearing impairment below 4 kHz (Table 1 detailed results in Appendix 1) Of the 79 patients studied 53 (67 per cent) had hearing impairment according to the above definitions. Sensory-neural impairments were clearly predominating, but some cases demonstrated impairments of a conductive or mixed type In the majority of cases the hearing impairment was moderate in fact most of the patients had not even observed a reduction in their hearing acuity

The most common type of *pure sensory-neural hearing impairment* observed was a characteristic basin-shaped threshold curve a dip (Fig 3) which was found in 33 patients (42 per cent) It was generally bilaterally symmetrical (22 cases) but sometimes unilateral with normal (five cases) or mixed (six cases) impairment on the other side The dip was generally located between 0.25–4 kHz, but could occasionally cover 0.125–8 kHz. The maximum impairment of the dip was almost exclusively found between 0.5–2 kHz, and the magnitude was 20–70 dB with a median value of 35 dB

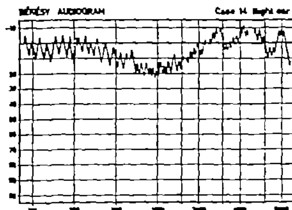


FIG. 3. Continuous recording showing the complete course of hearing threshold with minimal middle frequency impairment which easily is missed in routine octave audiometry

Recruitment was found in all these cases (Fig 4) except one which was questionable. Thus, the impairment is clearly of cochlear origin.

Nine patients (11 per cent) showed bilaterally symmetrical sensory-neural hearing impairment of other types (flat loss, sloping or high tone loss) which also demonstrated recruitment

There remain no doubts that patients with Turner's syndrome demonstrate sensory neural hearing impairment of a specific type—a dip—in a proportion far above what is observed in the general population. According to Anderson & Wedenberg (1968, 1969) dips of the same type occurred in 0.8 per cent of the boys and 0 7 per cent of the girls in a population consisting of 10 778 healthy school children (5623 boys and 5155 girls) around the age of 14 years.

Eight patients (11 per cent) demonstrated hearing impairment of *pure conductive type* six of which were unilateral and two bilateral Another nine (12 per cent) patients showed a *mixed type* of impairment, six unilateral and two bilateral In most of these cases, the audiometric pattern suggested an involvement of an underlying sensory neural dip. Conductive and mixed hearing impairment occurs only in about 1.6 per cent of healthy children around 14 years of age (Anderson & Wedenberg, 1969)

In a few cases the parents were studied (both in cases 11 17 24 37 and 75 only the mother in nos. 28 and 76 and only the father in no 19) and most of them were normal and no hearing defects similar to those found in the daughters were observed. Interviews with the patients and their parents did not indicate an increased frequency of hearing impairment among the close relatives (grandparents and their descendants)

It should be pointed out that none of the children studied before 10 years of age demonstrated hearing impairment. Between 10–19 years of age 20 out of 37 patients (54 per cent) between 20–29 years 17 out of 23 (74 per cent) and above 30 years seven out of nine patients (78 per cent)

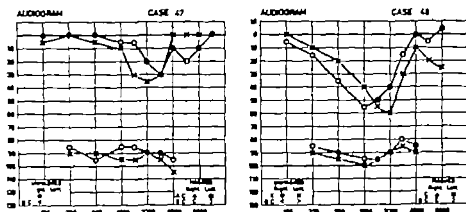


Fig. 4 Octa udiograms i two Turner cases showi g pronounced heari g threshold dips nd refl x threshold (dash-d tted) within normal limit ; the reduced span between heari g nd reflex threshold i dicates recruitment.

demonstrated sensory neural hearing impairment (Table 1) Twenty three patients were studied on several occasions, the intervals varying between one to eight years In six of these patients a progress of the sensory neural impairment of 10–30 dB was noted On the other hand, the mean depth of the dips did not vary significantly between different age groups. Thus, the hearing impairment generally seems to appear around or after ten years of age and then remains stationary or progresses very slowly

Hearing impairment between 4–8 kHz Hearing impairment above 4 kHz is much more difficult to measure and classify and there is no suitable Swedish control material Therefore, only a brief summary will be given and no detailed results have been included in Appendix 1 At 4 kHz 32 per cent of the patients showed hearing impairment of 20 dB or more, 18 per cent showed 30 dB or more The corresponding values at 6 kHz were 53 and 29 per cent, respectively There were no differences between the ears. There is a clear progression of hearing impairment with age and according to Glorig (1957) females between 30–39 years, show in 25 per cent an impairment greater than 10 7 dB (right ear)–22 2 dB (left ear) at 4 kHz. The corresponding values for 6 kHz were 28.2 and 28 0 dB respec tively If this material is used for comparison, our Turner patients have a clear hearing impairment also above 4 kHz. The occurrence of high tone loss is greater in the Turner group and only nine patients were 30 years or more A more detailed comparison could not be made due to the presenta tion of the control material

The cochlea was studied on X-ray films in ten cases (nos. 14 15 20 26, 29 40, 54 56, 63, 79) using tomography at 3–4 mm intervals Nothing abnor mal was observed in any of the patients. Furthermore an attempt was made to study the cochlea electronmicroscopically in cases 2 and 73 who died during the course of this investigation. It failed, however because of too pronounced autolytic changes.

Table 2 *Cephalometric indices, lateral projection*

The nomenclature used is described in Fig. 1. Significance of difference −P < 0.01

	Turner' Syndrome (I)			Normal subjects, age: 11 years (II)			Normal subjects, age: 16–25 years (III)			Difference between means		
Indices	n^a	mean	s.D	n	mean	s.	n	mean	s.D	II I	III I	III II
$(s\text{-}pe_1)$ 100 / (−)	45	30.4	4.9	33	31.5	3.7	46	32.1	4.5	1.1	1.7	0.6
$(s\text{-}pe_1)$ 100 / ($-pe_1$)	44	94.9	12.4	33	94.4	9.5	46	91.1	9.3	−0.5	−3.8	−3.3
$(po\text{-}pe_1)$ 100 / (−)	44	17.5	6.5	33	17.9	3.3	46	16.6	4.4	0.4	−0.9	−1.3
$(po\text{-}pe_1)$ 100 / $(pe\text{-}pe_2)$	45	90.6	52.1	33	91.7	23.6	46	118.8	60.8	1.1	28.2	27.1

$(s\text{-}pe\ )$ and (−) could not be measured in on case

CEPHALOMETRIC MEASUREMENTS

The results are given in Table 2. There was no difference between the patients and either of the control groups with regard to the *horizontal position* of the external auditory meatus.

In *vertical direction* the distance between the external auditory meatus and the SNL-line (*po-po*) relative to the height of the brain case (*v-s*) did not differ between any of the groups. However (*po-po*) was relatively larger than the distance between the external auditory meatus and the NL-line ($po\text{-}po_2$) in the patients than in the controls of the same age, *but not* in comparison to the controls of the same body height. Thus, the external auditory meatus is caudally displaced in Turner patients which explains the clinical observation of low-set ears.

It is known that the brain case grows very little from the beginning of puberty but that the facial skeleton grows markedly mainly in vertical direction This can for instance be visualized as a lowering of the nasal floor (*NL*-line) *i.e* a relative increase of ($po\text{-}po_2$) The development of the facial skeleton in adult patients with Turner s syndrome is impaired and only reaches a level corresponding to that of 11 years old normal girls (Filipsson, Lindsten & Almqvist, 1965) This impaired development of the facial skeleton might explain why ($po\text{-}po_2$) was found to be relatively shorter than (*po-po*) in the patients than in the controls of the corresponding age but not than the 11 years old controls of the same body height

No significant differences were observed with regard to the position of the external auditory meatus between groups of patients with different types of hearing impairment or different sex chromosome constitutions.

Table 3 *Cephalometric measurements frontal projection*

The nomenclature used is described in Fig. 1 N significant differences.

Measurements	11 patients with Turner' syndrome mean	s.d.	11 normal females mean	s.d.	Difference between means
linear mm					
(*co-cl*) left	16.2	2.7	16.2	2.9	0.1
(*co-cl*) right	15.7	2.7	15.9	2.4	0.2
angular degrees					
(*co-cl*)/OL left	24.9	5.7	28.2	5.0	3.4
(*co-cl*)/OL right	26 4	6.6	26.8	6.0	0.4

As seen from Table 3 there were no differences between the patients and the controls as far as the slope and length of the external auditory meati are concerned

STUDIES ON THE VESTIBULAR FUNCTION

Patients nos. 11 15 20 23 26 41 52, 53 and 66 were studied with regard to the vestibular function All patients studied demonstrated a sensory neural hearing impairment except one (no 23) whose hearing was normal Hyporeflexia was noted at the caloric reaction in two patients (nos. 15 and 66) one of whom also had spontaneous nystagmus (no 66) Case 20 also had spontaneous nystagmus, but reacted normally Preponderance was noted at the rotatory test in case 15 and secondary nystagmus in case 52.

Similar findings are frequently made in the general population Thus, the Turner patients did not demonstrate any striking abnormalities in the vestibular function.

DISCUSSION

The present work clearly confirms the preliminary observation by Lindsten (1963) that patients with Turner s syndrome demonstrate hearing impairment in a markedly increased frequency The most common type of impairment is of a specific type a bilaterally symmetrical sensory neural dip in the middle frequency range, but other types of impairment were also found occasionally Some patients have a combination of sensory neural and conductive i e mixed type of impairment Hearing impairment has earlier been occasionally reported in patients with Turner s syndrome and has generally been termed congenital unilateral or bilateral deafness (review in Hauser 1961 Silver & Dodd 1957 Haddad & Wilkins, 1959 De la Cha

pelle & Hortling, 1960 Slater & Zilkha, 1961 De la Chapelle, 1962 Josso, De Grouchy Frézal & Lamy 1963 Pitis, Stanescu Florea, Ionescu & Poenaru, 1963 Engel & Forbes, 1965 and Hugh Jones, Wallace Thornber & Atkin, 1965) The presence of a dip in some patients with Turner's syn drome has been confirmed by Giaccai, Zurli, Ricci & Piolanti (1966) Thus, even if the hearing impairment is found over the whole frequency range, the most characteristic change is the dip in the middle frequency range.

Patients with Turner's syndrome might have an increased frequency of repeated middle ear infections (Lenz, 1957 Silver & Dodd, 1957 Kaijser Söderhjelm, Enell & Kinch, 1959 Lindsten, 1963 Engel & Forbes, 1965 Stratton, 1965 and the present work) and conductive and mixed hearing impairment is clearly overrepresented This is probably due to changes localized to the ear itself *Other infections do not seem to occur in an increased* frequency and there are no clear indications of a defect in the immune response The growth disturbance in the cranial base including the temporal bone as demonstrated in the present work and by Filipsson, Lindsten & Almqvist (1965) probably causes an abnormal orientation of the middle ear and Eustachian tube which might predispose to the ear infections. Defects in the mucous membranes can of course theoretically do the same. Middle ear infections can in our opinion explain only the conductive type of hearing impairment but not the commonly observed sensory neural types, especially not the dip

There are no reasons to believe that other exogenous factors like asphyxia or intracranial haemorrhage at delivery or noise are of any significance in this connection Turner patients demonstrate a lower than normal birth weigth and a slightly shorter than normal gestation time The deliveries are almost exclusively normal (Lindsten, 1963) and all infants studied in the present work had a normal hearing. Our patients with Turner's syndrome, generally below 30 years of age had hardly been heavily exposed to noise. Even if they were considerably more sensitive to noise than normal this would *mainly explain the impairment above 4 kHz, but not the dips below* this frequency The conclusion will then be that both the typical sensory neural hearing impairment below 4 kHz and the impairment above this frequency are mainly of endogenous origin.

The use of the stapedius reflex test in recruitment diagnosis is based on the fact that in cases of recruiting hearing loss, the elevation of the hearing threshold is not accompanied by a corresponding elevation of the reflex threshold, i.e *the distance* between the two thresholds is reduced. In contrast, cases of non recruiting impairment demonstrate elevation of both the hearing and reflex thresholds. Although recent data has thrown some doubt on the absolute validity of this principle (Anderson & Wedenberg, 1968 *b*) this hardly affects the interpretation of the results in the Turner cases where the outcome of the reflex test with a few exceptions unequivocally indicated the presence of recruitment.

The rather uniform group of bilaterally symmetrical dips turned out to

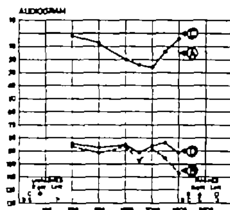


Fig. 5. Thresholds of hearing (*A*) nd refl xes (*B*) in charact ristic group f T rners (7 cases, 14 ears) nd correspo ding normal curves f hearing (*C*) nd refl es (*D*). Not divergent courses of hearing and reflex thresholds in upper frequency rang abov 2 kHz for the Turn gro p.

be of special interest since the reflex thresholds did not reflect the charac teristic appearance of the hearing threshold Consequently the impairments were judged to be recruiting In addition an elevation of the reflex threshold was commonly found at the highest test tones (3 and 4 kHz) i.e. well above the center of the impaired area, where the hearing threshold curves were normalized This peculiarity was more or less pronounced in the indi vidual cases, but the tendency was quite noticeable as seen from the median of the reflex thresholds for the seven most characteristic cases of dips submitted to reflex measurement (Fig. 5) The figure also contradicts the possibility that this phenomenon can be regarded as a simple function of the relatively small high frequency hearing threshold loss. The divergent course of the hearing and reflex threshold above 2 kHz in Turner cases is rather unique and no explanation to the phenomenon can be offered at present.

The fact that the dips almost exclusively demonstrated recruitment for mally localizes the defect to the outer hair cells of the organ of Corti It then remains to be shown why the dip is localized to the middle frequency range especially to 2 kHz. Judging from the experiments of Békésy (1960) this suggests a defect restricted to a comparatively short distance of the basilar membrane at the end of the upper and of the basal and at the beginning of the middle coil of the cochlea This part is of special interest since it has been shown that in the opossum (Larsell, McCrady & Larsell, 1944) as well as in the cat (Bosher & Hallpike 1964) and the mouse (Lorente de Nó, 1933) the embryological development of the receptors of Corti's organ starts in this region In man it is not yet known where this development starts, but there are no reasons to believe that man differs from the other mammals in this respect.

The histological studies just described are supported by physiological

observations in mammals during foetal life The first electrical responses from the organ of Corti can be obtained in the opossum within the frequency range 1–13 kHz (Larsell McCrady & Larsell, 1944) and in the rabbit within 2–5 kHz (Änggård, 1965) The first autonomic responses to acoustic stimuli appear in human foeti in the frequency range 1–3 kHz (Johansson, Wedenberg & Westin, 1964) These frequencies correspond to a stimulation area in the middle of the cochlea, in man to the upper part of the basal and the lower part of the middle coil. It is not known why this developmental region would be especially predisposed to hearing defects in patients with Turner's syndrome An embryological maldevelopment seems less likely since all the Turner infants demonstrated normal hearing The picture rather indicated a degeneration of sensory cells after the age of about 10 years. A longitudinal study of the hearing starting with newborn Turner patients might give further information regarding the pathogenesis of the sensory neural hearing impairment as might a histological and/or electronmicroscopical analysis of the cochlea in abortuses and perinatally dead children with a 45,X chromosome constitution.

It seems reasonable to assume that the abnormal sex chromosome constitution *is the etiological factor to the hearing impairment in patients with* Turner's syndrome X-linked gene(s) or an unspecific genetic imbalance would then be the most obvious alternative explanations. The dips observed in our patients are indistinguishable from those presumed to be determined by an autosomal recessive gene in heterozygous condition (Anderson & Wedenberg, 1968) In contrast to the Turner patients these cases frequently demonstrate, in addition to the dip, an abnormally high reflex threshold and a family history of hearing impairment.

We are then left with the hypothesis of a genetic imbalance which is *supported by the observations of abnormalities in morphology and function* of probably all organ systems (review in Lindsten & Fraccaro 1965) As far as the nervous system is concerned different neurological symptoms (Lindsten, 1963 and the present work in 14/75 patients) abnormal electroencephalograms (Mellbin, 1966 and the present work in 13/20 patients) and abnormal vestibular function (Slater & Zilkha, 1961 Giaccai, Zurli, Ricci & Piolanti, 1966, and the present work in 2/9 patients) have been described Furthermore the so-called "space-formed blindness" observed at the intelligence testing points in the same direction (e.g Money and Granoff 1965) In this connection it is worth mentioning that *there were no obvious* differences between groups of Turner patients with different sex chromosome constitutions.

The specific dip pattern, although less pronounced in five out of eight patients with Klinefelter's syndrome and an abnormal sex chromosome constitution (Anderson, Lindsten & Wedenberg, 1969) raises once more the question if there are specific factors on the human X chromosome influencing the hearing, or if certain parts of the cochlea are predisposed to changes in patients with unbalanced sex chromosome constitutions.

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APPENDIX

Middle ear infections audiometric and neurological analysis in 70 patients with Turner s syndrome classified according to sex chromosome constitution and age at examination

Case no.	Chromosome constit tion[a]	Initials[b]	Age t exami nation, years	Middle ear in-fections[c]	Audiometric analysis: Method[d]	Audiometric analysis: Type of impairment < 4kHz	Cephalo-metric analysis[f]	Neurological symptoms	Electroencephalogram
1	45 X	F H.	2/52	–	W	—	●	—	●
2		L S.	2/52	–	W	—	●	—	●
3		A. B.	3/52 2 /12	–	O	Probably normal	●	Delayed psycho-motor development epileptic seizures	At two and three months f age the EEG showed a right-sided slow wave-abnormality At five months the pattern had changed into a *marked and generalized* epileptogenic abnormality char cterized as modified hypsa-rhythmia. At six months these changes has regressed.
4		F E.	1 /12 2 9/12	–	O	—	●	—	—
5		K. B.	1 /12–3 9/12	+	O	—	●	●	—
6		M. G	/ –4	–	O	—	●	●	●
7		H G	1 / 3 9/12	+	O	—	●	—	●
8		S. B.	1 10/ –4 /12	+	O	—	●	—	—
9		A. M.	10	+ +	O	Right. mixed, flat – 60 dB Left. sens, dip 0.5–3 kHz Max, 2 kHz – 25 dB	+	—	●
10		B. E. A.	12 17	+ +	O	Right. sens, dip 0.25–4 kHz M x, 1 kHz – 10 dB Left. mixed, flat – 60 dB N progress	●	—	●

					ment. N progress			
12	L. A L.	13	+	O	Bil, sym, sens, dip 0.125–2 kH Max, 0.5 kHz – 25 dB	●	—	●
13	A Fn.	15-17	+ +	O	Bil, mixed: Right: sloping bove 1 kH 10–60 dB Left flat 25 dB No progress	●	—	●
11	G. B	16	+	OBR	Bil, ym, sens, dip 0.75–2 kHz Max, 20 dB t 1 kHz. Recruitment?	●	—	Slight generalized and diffuse slow-wave-abnormality
15	M. I J	15	–	OBR	Bil, sym, sens, dip 0.5–4 kHz Max, 2 kHz 20 dB. No recruitment	●	—	Slight generalized and diffuse abormality consisting of mi ture f fast and slow wave-activity
16	L M. I	15	–	OBR	Bil, ym, sens, dip 0.25–1 kH Max, 2 kHz 25 dB. Recruitment	+	—	●

N menclat re f the Chic g Conference (1966) Xqi = pres mp- ti i ochromosome for th l g rm f X. ? = nother celltype w pected ince the patient w re se hromatin positiv i ll f buccal m cosa. This celltype could, h ever not be found t th hi mosome naly i f blood cells. Xp – = probabl d l tio f the hort rm f X. X = ri g hromosom f X. Xq – = deletion f th l g rm or isochromosome for the short rm f X. m = centric fragment

Asterisk denotes patient tudied earlier from di logic l nd cytogenetical poi t f l w (Li dsten 1963)

– = N ea l fectio ; + = one a few ea l fections f whi h the patient had bee tre ted by physici n, pont neous perforati f the ear drum or paracenthesis; + + = se eral ar l fections for whi h th patient had bee hospit lized t l st nce and f which som had bee operated pon

O = Oct v diogram; B = conti ous frequ ncy a diometry accordi g t Békésy; R = t pedi reflex meas rement; W = h ri g test for l f t (see t t)

– = Withi norm l limit ; kH = test t e frequency i cy les per second di ided by 1000 dB = decibel bo e normal threshold f h ring; bil, sym = bilateral symmetrical threshold rve withi devia ti ns 10 dB less between ears i the frequency region 0 25–4 kH ; dip = ba l sh ped threshold curv figures f ll wi g denot frequency rang f basi t t; fl t = thresh ld curv reasonably h riz tal withi 0.25–4 kH ; sl pi g = threshold curv gradually f lling to- w rds higher freq nci (dB-figures d t begi ning nd ma im m f heari g loss ra g); sens = i.e he ri g impairment f pure sensory eural origi ; mi ed = mi ed hea i g loss, i.e heari g impairment in which both ensory eural d co d ctive compo ts re i lved; M x = ma im m; recruitme t = see t t for pla ti ; rising = threshold curve gradually risi g t ward higher frequencies.

⁽+ = Studied; ● = not studied.

– = N triki g bnormalities; ● = not studied.

Case no.	Chromosome constitution	Initials	Age at examination years	Middle ear infections	Audiometric analysis: Method[e]	Audiometric analysis: Type of impairment < 4 kHz	Cephalometric analysis[f]	Neurological symptoms[g]	Electroencephalogram[g]
17	45,X	L. B. J	10–18	–	O	Bil, sym, sens, dip 0.25–4 kHz Max 1 kHz – 13 dB No progress	+	Slight spontaneous nystagmus	Moderate generalized slow wave-abnormality sometimes of an episodic character and with changes most marked in temporal and central regions
18		F B.	17	–	OBR	—	●	—	●
19		M B.	17	+	O	—	+	—	—
20		K. M.	16	–	O	Bil, abrupt high tone Loss above 3 kHz	●	–	●
21		E. N.	18	+	O	Right. —			
						Left cond, max, 55 dB fixation of the ossicular chain	●	—	●
22		L. J	18	+ +	O	Right. cond, flat 50 dB	+	—	●
						Left —			
23		K. A	18	–	O	—	+	—	●
24		B. H.	18–20	+	O	—	+	—	●
25		D F	18–22	–	O	Right. —	+	—	●
						Left sens, dip 0.25–4 kHz Max, 1 kHz – 55 dB Progress 10 dB at 1 kHz			
26		L O.	19	–	OBR	Right. —	+	—	Slight diffuse increase of slow frequencies in frontal and temporal regions, possibly within normal limits
						Left sens, dip 1.5–6 kHz Max, 3 kHz – 30 dB. Recruitment.			
27		L. L.	19	–	O	—	+	—	●
28		M O	19	+ +	O	Right —	+	—	●
						Left cond, flat – 25 dB			
29		L L.	20 23	–	OBR	Bil, sym, sens, dip 0.25–3 kHz Max, 1 kHz – 40 dB. Recruitment No progress	+	—	Slight, diffuse increase of slow wave-activity especially in frontal and temporal regions with slight left-sided predominance

30	45,X	B. L.	21	+	O	Bil, sym, sens, dip 0.125–4 kHz Max, 1 kHz – right 15 dB, left: 30 dB	+	—	●
31		K. S.	21	+ +	O	—	+	—	●
32		L. A.	23	+ +	O	Bil, sens, dip 0.25–3 kHz Right. max, 1 kHz – 15 dB Left. max, 2 kHz – 40 dB	+	—	●
33		I. H.	21	+	OR	Bil, sym, sens, dip 0.125–4 kHz Max, 0.5 kHz 55 dB. Recruitment	+	—	Moderate diffuse slow wave-abnormality most marked posterior and lateral regions
34		S. I. H.	21	+ +	O	Right: mixed sloping – 40–80 dB Left. sens, dip 0.125 4 kHz Max, 2 kHz 55 dB	+	Spontaneous nystagmus	●
35		V. S.	20	+	O	Bil, flat, mixed. right 10 dB left 30 dB	+	—	●
36		R. L.	27	+	O	—	+	—	●
37		G. A.	28	+	O	Bil, mixed: right dip 0.25 4 kHz Max 1 kHz 55 dB Left. sloping 20 65 dB	+	—	●
38		G. K.	29–33	+	OBR	Bil sym, sens, sloping above 1 kHz 0–75 dB. Recruitment. Progress right 10 dB at 4 kHz and left 20 dB at 6 kHz	+	Ptosis right	●
39		M. S. L.	30	+ +	O	—	●	Strabismus	●
40		A. F.	31	+ +	OR	Bil, sym, sens, dip 0.25–2 kHz Max, 0.5 kHz – 20 dB. Recruitment	+	Intracerebral cyst, strabism	●
41		U. O.	32	+	O	Bil, sym, sens, flat. 20 30 dB	●		●
42		V. P.	31	+	O	Bil, sym, sens, dip 0.25–4 kHz Max, 2 kHz 35 dB	+	—	●
43		I. J.	41–42	●	O	Right: mixed flat 60 dB Left sens, dip 0.125–4 kHz Max 1 kHz – 70 dB Progress left 20 dB at 6 and 8 kHz	+	—	●

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S U P P L E M E N T U M 48

THE STAPEDIOVESTIBULAR JOINT
NORMAL STRUCTURE AND
PATHOGENESIS OF OTOSCLEROSIS

RUTH GUSSEN

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SUPPLEMENTUM 248

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THE STAPEDIOVESTIBULAR JOINT NORMAL STRUCTURE AND PATHOGENESIS OF OTOSCLEROSIS

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CONTENTS

REVIEW OF LABYRINTHINE CAPSULE STRUCTURE, NORMAL AND ABNORMAL

Otosclerosis occurs most frequently in the anterior portion of the labyrinthine capsule in relation to the oval window. Any theory of the pathogenesis of otosclerosis would have to account for the predilection of this area for the disease and yet still be consistent with the pathogenesis of the disease as described elsewhere in the bony labyrinth.

In a previous study (Gussen, 1968 *b*) it was shown that, although the labyrinthine capsule is preformed in cartilage, only small portions of the cartilage undergo bit by bit replacement by endochondral bone (globuli ossei). The great bulk of the cartilage degenerates and is replaced by marrow. This marrow is then gradually filled in by bone which is not endochondral bone since it is not formed by a bit by bit replacement of degenerating cartilage. The replacement bone is formed by mesenchymal cells within the marrow which transform into osteoblasts. This new bone is endosteal membrane bone which encases the scattered endochondral globuli ossei of the middle layer. The marrow spaces, after being largely filled in with endosteal convoluted membrane bone, remain as the blood vessel canals. The labyrinthine capsule therefore is essentially a membrane bone with scattered foci of endochondral bone (globuli ossei).

To understand the bone of the labyrinth, one must study the characteristics of membrane bone in general. One such characteristic of membrane bone is its ability, under certain conditions, to form cartilage. This cartilage, formed by cells that normally form bone, has been referred to in the literature as secondary cartilage or chondroid cartilage. An important characteristic of secondary cartilage is its ability to be converted into bone by two different processes. One process follows the usual degeneration of the cartilage with its bit by bit replacement by endochondral bone; the other is a direct transformation of the cartilage cells and the cartilage matrix into bone cells and bone matrix without degeneration and resorption of the cartilage, resulting in membrane (chondroid) bone. This type of bone forms most of the inner endosteal circumferential lining of the bony labyrinth.

It was demonstrated that secondary cartilage foci form throughout life from the mesenchymal cells adjacent to the inner lining of the bony labyrinth. These cartilage foci then either (1) remain as cartilage foci, or (2) undergo degeneration and replacement by endochondral globuli ossei, or (3) undergo direct transformation to bone as the inner endosteal circum

in its normal physiological manner due to the increased mineralization present within the canaliculi and lacunae which normally are free of mineral The micropetrotic perivascular bone may then break down by osteoclastic resorption and lacunar erosion The eroded bone areas are filled with mesenchymal tissue containing mesenchymal cells which form new bone as a repair mechanism This new bone forms as membrane bone within mesenchymal or fibrous tissue and represents "otosclerotic" bone

To be consistent, therefore a theory of pathogenesis of otosclerosis originating in the stapediovestibular joint region should involve the same principles of new membrane bone forming within the fibrous areas of eroded bone. In addition, the predilection of the joint area for this otosclerotic process must be explained.

THE STAPEDIOVESTIBULAR JOINT— A REDEFINITION

The author wishes to present a more composite anatomical and functional view of the stapediovestibular joint Descriptions of the joint usually refer only to the actual articulation between the stapedial footplate circumference and the oval window by means of the annular ligament Yet, Richany Anson & Bast (1960) and others have described the continuation of the cartilage lining from the articular portions of the joint to line the immediately adjacent areas of the middle ear and vestibule, and continuing also to line the fissula ante fenestram and the fossula post fenestram (when present) as well as the vestibular surface of the footplate itself

Brunner (1954) has described fibers of the annular ligament joining the connective tissue of the fissula ante fenestram Wolff & Bellucci (1956) concurred with Brunner's observation, describing the continuation of the fibers of the annular ligament into the fissula ante fenestram for their attachment They also observed that the fibers extend posteriorly to the fossula post fenestram and stapedius tendon and are continuous with the attachment of the spiral ligament fibers of the basal cochlear turn

The stapediovestibular joint, therefore may be viewed in a much broader sense as having an articular portion and a non-articular portion The articular portion refers to the direct stapedial footplate—oval window attachment by means of the annular ligament, and the non-articular portion refers to the cartilage-lined areas of the immediately adjacent tympanum and vestibule together with the fissula ant fenestram and the fossula post fenestram This broader interpretati n is believed to be tenable because of the directly continuous cartilage lining the articular and the non-articular portions, and because of the fiber connections from the annular ligament which, in their broad att chments and insertions serve for a more secure anchorage of the joint during m tion. The fi ula nte fenestram and the fossula post fenestram will therefore be con idered to be fibrous extensions of the stapediovestibular joint, pro id ng for additi nal broad anchorage of the footplate and tabilizing the joint

THE NORMAL STAPEDIOVESTIBULAR JOINT

Human temporal bones from 30 patients (51 specimens) were studied, ranging in age from birth to 82 years of age. Sixteen of the patients were males, from one day to 79 years of age, and fourteen of the patients were *females, ranging in age from 16 minutes to 82 years of age.* Two of the specimens contained otosclerotic foci involving the oval window without ankylosis of the stapediovestibular joint and were from a 47 year old white male. Four specimens were from Negro males, one pair from a one day old baby and the other pair from a 48 year old male.

The temporal bones were fixed in 10% neutral buffered formalin, demineralized by chelation with a 0.7 *M* solution of the tetrasodium salt of *EDTA at pH 7.4 and 37 C and vacuum embedded in parlodion.* Sections were cut at 20 micra and were stained with hematoxylin and eosin.

The normal histological structure of the stapediovestibular joint has been presented in a previous study (Cussen 1968 *a*). The cartilage lining the footplate, the oval window, the fissula ante fenestram and the fossula post fenestram is normally uncalcified. At all ages, varying degrees of degeneration of the chondrocytes within the uncalcified cartilage was demonstrated *with the presence of small numbers of macrophages within some of the* involved cartilage lacunae. The cartilage adjacent to the bone of the footplate, oval window and fissular tract undergoes a slow process of endochondral bone replacement throughout life. Once the macrophages have phagocytized the degenerated cartilage cells, osteogenic vascular buds extend from the underlying bone into the opened, excavated, "prepared" cartilage lacunae (Fig. 1). At times, endochondral bone gradually replaces large *areas of the cartilage of the vestibular surface of the footplate.*

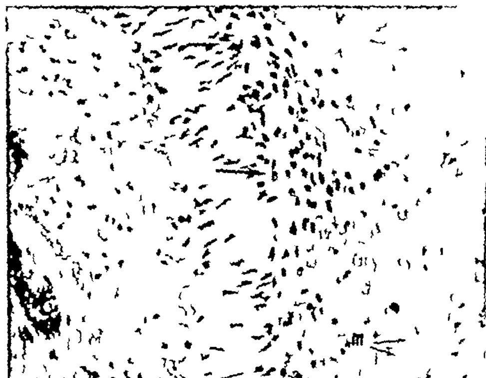
While endochondral bone formation is occurring in the deep portions of the cartilage of the articular and non-articular portions of the joint, the surface cartilage of the joint undergoes a constant repair and replenishment of its cartilage by mesenchymal cells within the annular ligament and fibrous extension of the joint. At all ages, but most prominent during the early active growth years, varying numbers of mesenchymal cells of the annular ligament *and fibrous* extensions are lined up alongside the cartilage surface becoming incorporated by cartilage matrix. Small surface defects in the cartilage are filled in or repaired by this process (Fig. 1). This process of continual cartilage degeneration and endochondral bone formation at the bone-cartilage junction, with new cartilage forming by apposi*tion on the articular surface, occurs at all ages, varying in degree after the*



FIG. 1 A (Reproduced from the American Journal of Anat my Gussen, 1968 a.) Posteri margin f val window N t pal degenerating chondrocytes nd scattered smaller irre gul rly shaped macrophages (m) w thi the cartilage la er and newly f rming chondrocytes t the cartilage surf ce Endochondral bo f rmation progresses from about the prominent blood vessel left f center On day ld male. H & E 280.



FIG. 1 B Ant rior margin of footpl te (right) and oval window (left) Not defect f rtilag of oval window (between rrows) with mesenchymal cell lined p longsid nd in parti l lacunae, forming new cartilage, in additio t cartilage repai t ll surf ces. Not dochondral bone formation i footpl t nd ov l window 3 yrs old f mal H & E 220.



F G. 1 C. Anterior margi f footpl t (l ft) nd al window (right) with nnular ligam t between. Note nse ca cartilag urfaces with shall w erosion f o l window larg singl arrow) and mesenchymal cells lined up longside forming new cartil g Deg nerating cartil g cell nd scall red macrophages (m) re present within the cartl lag 8-yea ld male H & E × 280



F G. 1 D Post m rgi of footpl t bo nd o l window (bel w Not newly forming cartilag ll from mesench m l cell f nnula ligament, nd degenerating pal cartil ge cel 4 ld f mal H & F 280

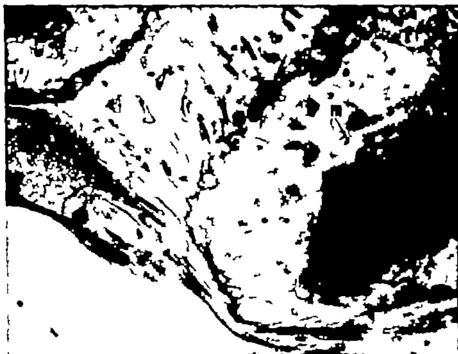


FIG. 1 *E* A terio margi of oval wind w (right) nd footplat (left) Note neven cartilag surface with degenerating cartilage cells, occasional macrophages (m) nd ew ndochondral bone formati n. Calcific tion i the rface cartil ge is noted i few places, as w ll as occa i nal new cartilage cells t the surface 79 year ld mal H & E 450



FIG. 2 Post rior margin of footpl t (right) nd oval window (left) Not *calcification* f cart l ge urfaces nd ll ea band f calcification in footpl te rticula cartilage t nd g t the nderlying bone Mesenchymal cell re ll ed p *alongsid for repai* Spac la lig ment l rtifact. 79 yea old mal H & E 130

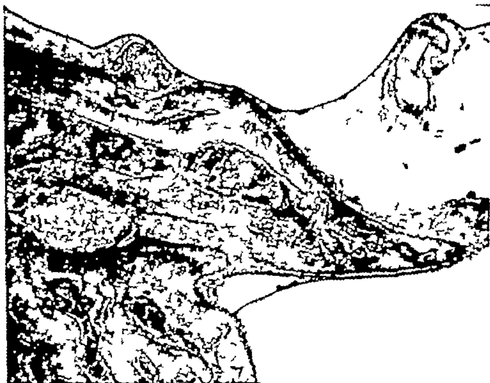


Fig. 3. A St pediovestibular joint in newborn f mal Not continuation f cartil ge lini g from ov l window (at right) t line the fissula nt fenestram. N t cartilag ma l deep part of fissula. Not abundant marrow within otic capsule replaced by membrane bone which encases the endochondral globuli ossei. H & E ×45.

active growth varies from specimen to specimen and appears to represent physiological repair and remodeling of the joint

After approximately the third decade the cartilage of the articular and non articular portions of the joint commonly reveal foci of surface calcification which may extend in narrow linear bands to the underlying subch ndral bone Fig 2 demonstrates both urface and linear bands of calcificati n in the posterior portion of the footplate articular cartilage of a 0 year old male Small erosions of the surface cartilage result from the breakdown of the calcified cartilage matrix, which is unable to maintain itself and mesenchymal cells form new cartilage (physiological repair) Figs. 3 B and 3 C demonstrate surface calcification and linear calcification f the cartilage lining the fissula ante fenestram

The fi ula ante fenestram in addition to the changes already described als revealed certain changes which were localized to that structure In virtually all pecimens, the deep portion of the fissula ante fenestram was n l nger lined b a cartilage layer but contained either a ma s of cartilage (Fig 3 A) r cartilag undergoing endochondral or membrane chondroid bone tran f rmati n, r was lined by bone (Fig. 3 B and 3 C) In all pecimen available t the author these changes were limited to the deep p rti n f the fi ula ante fenestram and did not fill the entire tructure



FIG. 3 B Fissula t fenestram (between arrows) with calcification of rtilage li ing deep porti nd filli g f deep porti with cartilage in transition t membrane chondroid bone N t linear band of calcification in cartilag lining i pper right field. 62 year old male H & E 130

Fig 3 A demonstrates the fissula ante fenestram in a newborn female The deep portion of the fissula is filled with cartilaginous tissue Fig. 3 B demonstrates the fissular tract in a 62 year old male. Here, the deep portion of the tract is lined by calcified cartilage and contains new matrix secreted by the mesenchymal cells which resembles an inbetween stage of cartilage-bone matrix. This represents the formation of membrane bone in this area which is going through a modified secondary cartilage stage Fig 3 C is the fissula ante fenestram in a 48 year old Negro male and demonstrates the deep portion asymmetrically filled with membrane chondroid bone Bast & Anson (1949) have described thi type of change in the fetus and young child, and interpret it as an attempt by the tissues to reduce the size of unusually large portions of the fissula The presence of cartilage and/or bone in the deep portion of the fissula is probably related to the constant motion of the fibers inserting in this area which are continuous with fibers in the annular ligament. This will be discussed more fully later The auth r believes that some degree of cartilage or bone formation within the deep portion of the fissula represents a physiological process of remodeling related to mechanical loading

Since foci of cartilage calcification and superficial erosion of the stapedio-estibular j int are present to uch universal degree, these chan... re



Fig. 3C. Fissula ante fenestram demonstrating asymmetrical Id membrane chondroid bone filling deep portion (arrows). Note calcification of surface cartilage. 48 year old Negro male. H & E × 130.

considered to be a part of the physiological wear and tear of the joint surfaces, with repair and remodeling occurring in the form of endochondral bone formation at the cartilage-bone junctions, and appositional cartilage formation at the articular surfaces. A sharp dividing line between the changes seen physiologically with function and age and the beginning changes of degenerative joint disease (osteoarthritis) is difficult to define.

The stapediovestibular joint, therefore, differs from the majority of joints in the body which are lined by typical hyaline cartilage and in which growth and repair of the cartilage occur by proliferation of cartilage cells deep within the cartilage layer. The stapediovestibular joint is not lined by this typical hyaline cartilage. In this respect it resembles such joints as the temporomandibular joint (Moffett Jr. *et al.*, 1964) and the sternoclavicular joint, which have in common repair of the articular cartilage surface by apposition of cartilage formed by mesenchymal cells in the adjacent fibrous tissue. Miles & Dawson (1962) point out that in the case of the temporomandibular joint, this has been attributed to the fact that the temporal and mandibular components of the joint are derived from membrane bone. The cartilage of the stapediovestibular joint is secondary cartilage (or chondroid cartilage) and, as has been demonstrated in previous studies, is associated with bone which has been formed predominantly as membrane bone (ussen 1968 *b*

ABNORMAL CHANGES
(DEGENERATIVE ARTHRITIS)

Occasional specimens exhibited deep erosion of the articular cartilage of the oval window and of the footplate Figure 4 *A* reveals such a deep erosion or ulceration of the anterior articular surface of the footplate, with calcification and superficial erosion of the cartilage lining of the fissula ante fenestram in a 75 year old white male The erosion of the footplate cartilage lining extends almost to the underlying bone. Only a thin, irregular rim of cartilage matrix remains separating the mesenchymal tissue filling the erosion and the subchondral bone. Severe degenerative arthritis also involved the stapediovestibular joint, bilaterally of a 57 year old white female Both articular and non-articular surfaces were affected. Fig 4 *B* demonstrates deep erosion of the cartilage of the anterior surface of the left stapedial footplate The surface of the remaining cartilage is calcified The apposing oval window surface and the fissula ante fenestram reveal thinning of the cartilage lining with calcification and mesenchymal cell repair Congested capillaries are present just beneath the middle ear mucosal surface of the annular ligament, presumably as part of the reaction to the articular degenerative changes. The eroded portion of the stapedial articular cartilage contains mesenchymal tissue in which mesenchymal cells and increased amounts of mucopolysaccharide material (from the degenerated cartilage matrix) produce an illusion of outline of the original uneroded footplate contour Fig 4 *C* is a high power view of the footplate erosion demonstrating the dense calcific material obscuring remnants of degenerating eroded cartilage. The mesenchymal cells and tissue within the erosion resemble fibrocartilage Fig 4 *D* reveals an eroded portion of the left oval window margin anteriorly at a slightly deeper level. Remnants of the cartilage lining are seen at the bottom of the photograph. Above this, the cartilage lining has been completely eroded with continuing erosion of the underlying bone which has now been exposed by the arthritic process. Mesenchymal cells are present within the pitted, irregular eroded surface of the exposed bone Fig 4 *E* demonstrates erosion of the posterior portion of the footplate articular cartilage in the right temporal bone Degenerating cartilage undergoing repair is present on either side of the erosion In the eroded area itself the cartilage has been completely destroyed and erosion of the subchondral bone is evident Mesenchymal cells are present within and alongside the eroded, pitted bone surfaces. Fig 4 *F* demonstrates erosion of the non-articular surface of the joint in the



FIG. 4 E Right stapediovestibular joint with calcification and marked thinning of foot plate cartilage posteriorly Note small deep erosion (arrow) of articular surface down to and involving the underlying bone Note mesenchymal cell within pitted, eroded bone surface as well as repairing cartilage at bottom of photograph. 57 year old female H & E 450

form of a large eroded area within the vestibular surface of the right foot plate adjacent to the articular surface Erosion of the underlying bone has occurred, and mesenchymal tissue fills the eroded area Mesenchymal cells are present alongside the pitted irregular eroded bone surface Such severe degenerative arthritis was seen in only a few specimens, and in no such case was any history of hearing disorder present



FIG. 4 F Right st pedial footplate with rticular surface (left) and estibular surface (bott m) Note large erosion of vestibular surface with erosion f nderlyi g bone (single rrow) Mesenchymal cells fill the erosion ca ity Note small irregul resorption cavity (small arrows) within articular cartilage 57 yea old female H & E 450

Temporal bones from one patient demonstrated very severe degenerative joint disease with deep erosion of the underlying bone and exhibited early new bone formation (repair) by mesenchymal cells. This new bone formed by the mesenchymal cells partially filled in the deeply eroded articular surface. Interestingly enough these bilateral changes of severe degenerative joint disease with resulting repair by new bone formation occurred in a three-year old white Mongoloid female with congenital heart disease. The etiology of the degenerative arthritis in this case is undetermined. As far as is known there were no symptoms referable to the ears. The patient died following corrective cardiac surgery.

Fig. 5 *A* demonstrates the articulation between the footplate and the oval window posteriorly. Extensive deep ulceration of the articular surface of the oval window is evident with complete destruction of the cartilage lining in this area and deep erosion of the underlying bone of the labyrinthine capsule. A narrow rim of newly formed bone is present beginning to fill in the eroded area. A few calcific plaques are present within the erosion remnants of the previously calcified cartilage. The articular cartilage of the footplate appears normal in Fig. 5 *A*. However, in Fig. 5 *B*, which is at a slightly deeper level, erosion of the apposing footplate cartilage is evident with mesenchymal cells lined up alongside the defect. Here cartilage is present along one edge of the large oval window erosion. New bone formation is evident beginning to fill in the deeper portions of the eroded area. Note the cellularity of the newly forming bone as compared to the adjacent older bone of the labyrinthine capsule. Fig. 5 *C* demonstrates erosion of both oval window and footplate articular surfaces posteroinferiorly. The oval window erosion is filled in by new bone at the edges of the erosion up to the original surface. However, the midportion of the erosion has not yet completely filled in with new bone. The photograph demonstrates well the sharp demarcation between the bone filling in the erosion defect and the uninvolved cartilage. The apposing footplate surface is eroded down to the underlying bone with clusters of mesenchymal cells within the area. Fig. 5 *D* is a high power view of the newly forming bone filling in the erosion defect in the oval window. The new bone is membrane bone formed by the mesenchymal cells.

Fig. 6 demonstrates a sclerotic replacement of the oval window without ankylosis in a 47 year old white male. It also demonstrates a deep erosion of the apposing cartilage surface of the footplate down to the subchondral bone. The annular ligament has been encroached upon by the

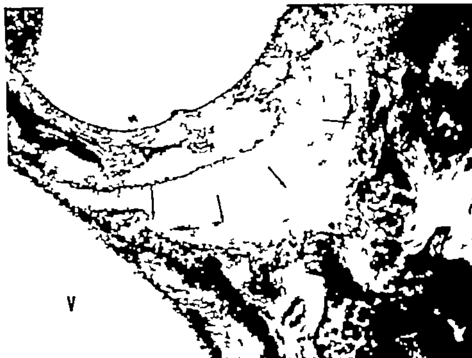


FIG. 5 A St pediovestibula j lat, posteri rly Not xtensi deep erosion f oval window surface (arrows) with complet destruction of rticular cartil g nd deep erosion f underlying bone Not irregula narrow rim of ew bone forming along eroded bone urface Calcific plaques are present i the eroded rea, rem ts f previousl cal ified cartilag V Vestibul 3 yea ld female. H & E ×125



FIG. 5 B. Slightly deeper tha bov Not cartil ge long left edg of rosion Not newly formi g membra bone rrows) begi g t f ll d p part of erosion. Not cell lar- it of ew bone compared t adjac t ld bone of l b l th ne capsule Not erosion f pposi g surface of footplat with cl t rs f mese h mal cells. H & E 125



FIG. 5 C. Newly forming membrane bone has completely filled in oval wind w erosion t l ft, and form val window urface (l ft arrow) At right newly formed bone is h rply dem rcated from unin lred rticular cartilage (right arrow) Between these tw points, the rosion i nly partially filled in with new bone Apposing footpl te ur face i eroded and underg ing repair H & E ×160

actively forming new bone of the oval window The spread of such actively forming new bone to the footplate does not appear to occur by invasion of the cartilage lining of the footplate Rather mechanical function of the j int is further impaired by the new bone repair of the oval window surface and results in degeneration of the footplate articular cartilage With deep erosi n of the footplate cartilage and eventual erosion of the underlying b ne of the footplate bone repair may ensue to fill in the defect in the f otplate which may gradually merge with the actively forming new bone of the oval window surface The process seems to be one of continuing degenerative arthritis and repair where the malfunction of the joint prepares the way for further erosion of cartilage and bone with resulting new b ne f rmati n

Thi new meml rane bone involved in the repair of arthritic lesions where er i n f ubch ndral bone has occurred, is identical to otosclerotic b ne Ot sclerotic bone per se is therefore not abnormal bone It is mem brane b ne f rmed by mesenchymal cells which normally form secondary cartilage in this area but which have the capability of f rming bone directly The erosion f the ubchondral bone stimulates the mesenchymal



FIG. 5 D High power view f newly forming membran bone filling I al window articular erosion. H & E 250.

cells to form bone as a means of repair A vicious cycle is then set up where increasing repair further increases the malfunction of the joint, which accelerates the degenerative lesions, requiring further repair

None of the specimens available to the author demonstrated complete filling of the fissula ante fenestram with bone However one can speculate that such extensive bony replacement of the fissular soft tissue represents a response of this area to mechanical overloading, exerted through the fibers from the annular ligament which insert deep within the fissula ante fenestram There is considerable evidence available in the literature to indicate that cartilage (and its conversion to bone) can be induced to form in connective tissue at points where there is movement combined with pressure or mechanical loading (Murray 1936 Ham & Harris, 1956) It is conceivable that when such new bone is found in excess within the fissular tract, that the oval window articular surfaces will demonstrate severe degenerative arthritic changes with possible deep erosion involving the underlying bone and beginning new bone repair (otosclerosis) Yet it would seem that arthritic lesions may involve the articular surfaces severely without necessarily overloading the fissular area. Much additional study of this area is necessary



FIG. 6 Oval wind w margin (l ft) l repl ced by otosclerotic bone which i tendi g through l lig m t t bott m. Not deep erosi (arrows) of ppo i g footpl t cartil g d wn t subch ndral bo 47 y ar ld mal H & E 150

DEGENERATIVE ARTHRITIS AND OTOSCLEROSIS

The stapediovestibular joint throughout life suffers the effects of physiological wear and tear and is able to maintain its articular surfaces by apposition of new cartilage formed by the mesenchymal cells of the annular ligament and fibrous extensions of the joint. The articular cartilage of the joint is normally uncalcified, but with increasing age small foci of calcification and surface erosions occur These foci of cartilage degeneration and erosion may increase in number and dimension and merge into the early stages of degenerative joint disease (or degenerative arthritis or osteoarthritis) Degenerative arthritis implies the degeneration of articular surfaces with continuing attempts at repair by the joint tissues. As the degenerative changes become more and more severe the mechanical function of the joint is further impaired, and, in turn, exacerbates the degenerative changes, producing a vicious cycle and acceleration of the degenerative arthritic changes. With focal erosion and destruction of the cartilage lining, the underlying bone is exposed and becomes eroded When this occurs, the mesenchymal cells within the annular ligament bordering on the eroded areas (and probably the mesenchymal cells about the blood vessels of the eroded bone) are stimulated to form new bone in an attempt to fill in the erosion defect. This new membrane bone, formed to repair the eroded bone, is identical to "otosclerotic" bone Otosclerotic bone therefore is not abnormal bone, per se It is membrane bone formed in an area of bone erosion, presumably to repair the defect Its formation in the stapediovestibular joint is analogous to its formation in the labyrinthine capsule proper in that, in both places it forms to repair areas of bone breakdown or erosion The breakdown or erosion of bone in the labyrinthine capsule proper is related to vascular insufficiency and micropetrosis (Gussen, 1968 *b*) whereas the erosion of bone in the joint area is related to severe degenerative joint disease Once the erosion or breakdown of bone occurs in either area, the repair process of new membrane bone (otosclerosis) formation follows. The predisposing factor in the joint region is the occurrence of degenerative joint disease.

Degenerative arthritis may result from many types of specific joint disease in addition to occurring as an independent entity Some of the specific disease processes in which degenerative arthritis occurs are certain of the osteochondrodystrophies, certain metabolic disorders such as ochronosis and gout, trauma, and irradiation When degenerative arthritis involves a joint where no specific etiology is recognized, the factor of

mechanical stress to that joint associated with such factors as genetic predisposition and hormonal influence is implicated

Mechanical Stress Associated with Genetic and Hormonal Factors One of the main factors implicated in the etiology of degenerative arthritis is mechanical stress. This raises the question of *what* constitutes mechanical stress to the stapediovestibular joint—a joint which is normally in constant motion Johnson (1959) in his discussion of the kinetics of osteoarthritis, states that tangential shearing forces are essential for the development and localization of the joint lesions. A shearing force implies a sliding contact of parts which move in opposite directions from each other The action of the stapedial footplate against the oval window fits into this category of tangential shearing motion However the manner in which mechanical factors operate is poorly understood, and other factors, such as genetic predisposition and hormonal influence are usually implicated as associated with the mechanical stress theory of degenerative joint disease

Age changes within cartilage matrix may well predispose to its mechanical erosion Silberberg & Silberberg (1941) studied the age changes of bones and joints in various strains of mice and found definite genetic differences in the rate of development and severity of such changes. Sokoloff *et al* (1962) found hereditary factors to be of major importance in the development of degenerative joint disease in various strains of mice Sokoloff *et al* (1967) also demonstrated a predisposition to degenerative arthritis specifically of the intervertebral disks in small rodents (Praomys Mastomys Natalensis)

A similar genetic predisposition has been demonstrated in humans with Heberden s nodes (Stecher 1940 Kellgren 1964) where the incidence of this degenerative arthritis of the terminal phalanges of the fingers is higher in women and genetically appears to depend on a single autosomal dominant gene whereas in men it appears to be recessive Approximately 25% of Caucasian women are predisposed to this form of osteoarthritis, whereas it is less prevalent among Negroes, Eskimos and possibly Japanese

Other factors, perhaps in conjunction with mechanical stress and genetic predisposition may be involved For example Sokoloff *et al* (1960) found in experimental studies in rats and mice that a high fat diet enhanced the joint degeneration in certain strains Articular cartilage responds to physiological stimulation in several respects. For example, Silberberg & Silberberg (1941) in their study of age changes of bones and joints in mice observed that the age changes occurred more slowly in males than in females, and that pregnancy exerted an accelerating effect. Excessive secretion of pituitary growth hormone results in acromegaly in the adult Hypertrophy of articular cartilage which then degenerates has been demonstrated in this condition, both in humans (Waine *et al* 1945) and experimentally in animals (Kellgren *et al* 1952)

It is interesting to note here that some of these factors predisposing to

degenerative arthritis are also factors which seem to have significance in the incidence of otosclerosis. For example otosclerosis has been considered to have a hereditary basis, the mode of inheritance being through an autosomal dominant gene (Larsson, 1960) The incidence of otosclerosis is also much higher in the white race than in the Negro and Oriental races (Guild, 1944 Altmann *et al.*, 1967) The hormonal effects of pregnancy on clinical otosclerosis are well known It may well be that these factors predispose the stapediovestibular joint to degenerative arthritis, setting in motion the events which may culminate in otosclerotic" membrane bone repair of a severely eroded articular surface

There are numerous studies and discussions in the literature on the possible role of mechanical stresses and strains in the development of otosclerosis (Mayer 1931 Sercer & Krmpotic 1966, and others) These studies discuss the mechanical factors resulting from extrinsic forces and resistance of the otic capsule or from rotation of the capsule during development or from the changing angulation of the base of the skull. Howeve hardly any reference is found to the more obvious mechanical factors involved through the motion of the *stapediovestibular joint itself* It would seem that the stumbling block has been the search for a common etiological factor for the development of otosclerosis in the joint area and the labyrinthine capsule proper rather than a common pathogenesis. The pathogenesis of otosclerosis is the same in both regions, provided that one starts with the *basic lesion of erosion or destruction of bone* Once this occurs, in either area, the resulting bone repair recognized as otosclerosis occurs. However the events leading up to the erosion of bone in the two separate locations, are different

Mechanical stress has been implicated in the pathogenesis of otosclerosis in the joint region in the past, but with no clear understanding of the normal and abnormal tis ue reactions in this area. For example Brühl s mechanical irritation theory as discussed by Mayer (Brühl, Mayer 1931) stated that mechanical irritation of the area anterior to the oval window esulted from the constantly moving annular ligament and the contraction of the tensor tympani muscle bringing about the formation of otosclerotic bone Brunner (1952) recognized degenerative changes in the cartilage of the oval window considering them to be "pre-otosclerotic These changes involved loss of nuclei and calcification. He described the changes as occurring normally to a certain extent, but occurring more extensively in pre-otosclerosis. Brunner believed that the degenerative changes were atrophic changes and were probably hereditary

Metabolic Factors Any mechanism interfering with the normal articular structure may lead to the development of degenerative arthritis. For example ochronosis is a hereditary disorder of metabolism of the amino acids tyrosine and phenylalanine which lead to the deposition of a pigment (homogentisic acid) in cartilage and certain collagenous tructures. The

homogentisic acid hyperpolymerizes the mucopolysaccharide of the cartilage matrix to a brittle glasslike consistency that is easily fractured This articular cartilage involvement results in the gradual development of degenerative joint disease (Johnson 1959)

Brunner (1929) has described the temporal bones of a 54-year old male with ochronosis. The patient died of heart disease, and also suffered from degenerative joint disease of the vertebral column Brunner's main interest in the temporal bones was the demonstration of the abnormal pigment An incidental finding was a focus of otosclerosis involving the oval window without ankylosis of the joint Unfortunately no photomicrograph of the focus is included in his presentation. However it is logical to consider that degeneration of the articular cartilage of the oval window had occurred secondary to pigment deposition, and that the otosclerotic focus represented a repair of a severely eroded articular surface down to and involving the subchondral bone Brunner did not see abnormal pigment in the cartilage however the cartilage in the involved area had probably been destroyed

Osteochondrodystrophies Severe precocious degenerative arthritis has been described in humans in association with various osteochondrodystrophies (Moldawer *et al.*, 1962) Certain breeds of dogs with inherited peculiarities of articular cartilage are prone to degenerative arthritis. Dachshunds, for example have a high incidence of degenerative intervertebral disk disease (Hansen, 1959) and degenerative arthritis of the hip is very common in certain breeds of dogs, such as German Shepherds where it develops secondary to dysplasia of the hip (Riser 1963)

An increased incidence of otosclerosis is found in some osteochondrodystrophies, and may well be related to degenerative changes involving the articular surfaces of the stapediovestibular joint, predisposing to erosion of the subchondral bone The dystrophic diseases all have in common some failure of matrix formation In Morquio's disease, for example, there is an abnormal accumulation of mucopolysaccharides in cartilage matrix resulting in degenerative arthritis. In Marfan's syndrome the basic lesion appears to be an over-accumulation of chondroitin sulfate followed by disruption of elastic fibers. It is not illogical to postulate a degeneration of articular cartilage of the stapediovestibular joint in either of these diseases. Once degeneration of the articular cartilage occurs, the likelihood of injury (erosion) to the underlying bone increases, with attempts at repair and the formation of new bone

Osteogenesis imperfecta has been the main osteodystrophy associated with otosclerosis. This disease is essentially a failure of periosteal and endosteal membrane bone formation (Rubin, 1964) Articular surfaces are usually described as normal in accounts of the disease although there is hyperlaxity of ligaments which leads to frequent dislocation. However it must be borne in mind that the cartilage of the labyrinthine capsule is not

primary hyaline cartilage, but is secondary cartilage formed by mesenchymal cells by apposition In other words, it is cartilage which is formed by cells that also normally form membrane bone The articular cartilage of the stapediovestibular joint is also secondary cartilage formed by apposition of mesenchymal cells This secondary cartilage formation therefore of the articular surfaces of the stapediovestibular joint, is analogous to membrane bone formation, in that the same mesenchymal cells are capable of forming either tissue This differs from the majority of the joints in the rest of the body which are lined by hyaline cartilage, and which maintain themselves by slow growth of cartilage from cartilage cells within the bulk of the cartilage itself and not by apposition. Since osteogenesis imperfecta is primarily a disorder of membrane bone formation (or a form of connective tissue failure) it is logical then that the secondary cartilage lining of the stapediovestibular joint which is formed by the same mesenchymal cells that form membrane bone —that the articular cartilage of this joint will be involved in defective formation with resultant predisposition to degenerative arthritic changes. This may be the explanation for involvement of such a joint as the stapediovestibular joint in the disease process and the sparing of the joints lined by typical primary hyaline cartilage It would be interesting in this respect to examine the temporomandibular and sternoclavicular joints in patients with osteogenesis imperfecta to determine if these joints, which are also lined by secondary cartilage react as does the stapediovestibular joint.

Paget's disease of bone represents an error in the remodeling of bone where both bone resorption and membrane bone formation are increased, with first one phase and then the other being more active The poor structural quality of bone in this disease predisposes it to injury In this regard, there is some impressive evidence (Sokoloff 1960) that erosion of articular cartilage is especially likely to occur in domesticated animals where there is insufficient mineralized bone tissue Again, one can also speculate on the involvement of the secondary cartilage lining of the stapediovestibular joint in the process, since its formation is analogous to membrane bone formation

Irradiation Recently the possible relationship of irradiation in the region of the ear to the subsequent development of otosclerosis has been raised. Kristensen & Jörgensen (1967) demonstrated such a possible relationship between otosclerosis and irradiation There have been numerous reports dealing with the effects of irradiation on cartilage cells and osteoblasts These are summarized by Gall *et al* (1940) and Heller (1948)

The early post irradiation changes in bone and cartilage have been studied by Melanotte & Follis, Jr (1961) They studied the effect of a single exposure of x irradiation of increasing intensities from 400 to 1800 r on epiphyseal cartilage and osteoblasts of the tibia in rats. Not only was the growth of cartilage affected but the cartilage cells were disorganized The

Solche Faktoren wie mechanische Belastung verbunden mit genetischer Veranlagung, hormonale und metabolische Faktoren (so wie Schwangerschaft und Ochronose) gewisse von den Osteochondrodystrophien und Ausstrahlung wurden in ihrer Rolle als Faktoren die empfänglich machen für die Entwicklung von degenerativer Arthritis besprochen im allgemeinen sowohl als auch in ihrer möglichen Rolle in der Entwicklung von degenerativer Arthritis des stapediovestibulären Gelenks (auf diesem Wege empfänglich machend für Otosklerose)

Osteogenesis imperfecta ist eine Krankheit der Membranknochenbildung Der sekundäre Knorpel der das stapediovestibuläre Gelenk auskleidet ist analog dem Membranknochen insofern als dieselben Zellbindegewebe beide Gewebe bilden Daher steht das häufigere Vorkommen von Otosklerose bei Patienten mit Osteogenesis imperfecta wahrscheinlich in Beziehung zu degenerativer Arthritis sekundär zu artikulärer Knorpeldefektbildung.

Man nimmt an, dass die neue Knorpel- und Knochenbildung die man gelegentlich innerhalb der fissula ante fenestram antrifft, sekundär eine gesteigerte mechanische Belastung dieser Struktur ist

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CONTINUED STUDIES ON THE FUNCTIONAL MECHANISM OF THE LABYRINTHINE SENSORY EPITHELIA

The Vestibular Part

particularly the Otolith Organs

S. H. MYGIND

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To the memory of Aug. Krogh, professor of Zoophysiology my moral support and source of inspiration.

While we elsewhere in sensory physiology admittedly have to do with influences acting in the axis of the sensory cells, producing partly a topographically localized sensation, partly oppositely directed motor reactions, serving the positional adjustment, as is the case in sight and sensation of touch, a corresponding mechanism of the labyrinthine senses, acoustic as well as vestibular is unanimously rejected in our days.

Quix maintained that the otoliths were acting by pressure. This conception was disproved by a series of ingenious experiments, carried out by Magnus and de Kleyn, apparently proving that the otoliths were acting by hanging (for details Mygind[4]). About the same time I had started working with clinical problems which I could not make to fit into the above theories. My first step into the right direction was a comparison with the eye where a stimulation of the one part of the retina releases an opposite feeling of orientation and an oppositely directed motor reaction in relation to the stimulation of the other part. The one side of the retina thus cooperates with the homolateral part of the other eye, while the temporal and the nasal parts of the single eye are oppositely orientated, as are also the upper and lower halves. I also recognized that the same place of a vestibular epithelium gives corresponding reactions of eyes, neck, body and extremities, reactions which, previously at least partially had been considered as originating in different epithelia. As incitament I accepted pressure because I in our Menièrepatients had observed so many phenomena indicating an existing intralabyrinthine over-pressure (D Dederding[1]). And I also found out, that clinical and experimental facts became explainable, if the sensibility throughout a sensory ephitelium was not, as hitherto supposed, equal all over the same surface, (S.H. Mygind [1 2 3-4]).

By studying the famous work of G. Retzius from 1881 and 1884 3 of the comparatively few microscopical preparates showed very interesting pictures of the ma-

cula utriculi in a cod-fish, an alligator and a pigeon. Both the cod-fish and the pigeon showed in a sagittal section an increase in height from behind forwards of the sensory cells, ending in front with a sudden steep fall, while the backward part showed evenly diminishing cells with no sharp frontier towards the surroundings. The upper surface was in the pike (fig. 1) somewhat excavated in the pigeon nearly horizontal and flat (fig. 2). Corresponding differences were seen in the alligator only it is not noted what is front or outside. In all 3 cases the part with the highest cells, what I have termed dominant, was supplied by the at the same time stronger and more peripheral part of the nerve while the opposite part, which I termed subordinate, was supplied by the weaker more central part.

But I realized that this rule of dominance was not only of greatest importance for the understanding of labyrinthine function but also a part of a general rule. The snout is thus more sensitive than the cheek, the finger-ends than the palm, the hand than the arm the arm than the shoulder the belly than the back etc. Often there is an intermediate neutral or blind part interposed between dominant and subordinate parts. In the eye there is the macula ceca, while the peripheral dominant temporal part holds the fovea. The temporal part also shows its superiority in certain transplantation experiments on newts by Stone. In the organ of Corti the roof of the tunnel forms the blind part separating between the inner subordinate with one range of hair cells and the outer dominant part with several ranges of larger cells.

It is the dominance difference which explains the special vulnerability of the base of the cochlea in contradistinction to the apex (»treble deafness») and of the internal in contradistinction to the external hair cells, as clearly seen in the streptomycin experiments of Engström and Engström and Kokonen as also in further intoxication experiments of Engström, Ades and Andersson.

I further drew the conclusion that the well known sensibility differences between the canalicular and the utricular surfaces and the opposite direction of this relation in the horizontal and in the vertical crests depend on a corresponding difference. This difference is directly visible only in the Myxine and perhaps in the Acanthias, as we later will see

How acceleration, rectilinear or curved acts by an alteration of pressure on a macula, respectively a crest, is illustrated in fig. 3 and 4 Gravity acts as a continuous perpendicularly downwards directed pressure.

The principle of oppositely orientated and reacting parts of static organs seems to be a very old one, as we refind it in the otolith organs of the tail-feet of the crustacian mysis (fig. 5) (v Buddenbrock).

Also the vestibular tonus reactions are pressure reactions. Quix believed this reaction to be due to the pressure of the heavy obolithes on the maculae. I referred it to a diffuse hydrostatic pressure of the endolymph on both maculae and crests. Ewald had thought it caused by a constant vibrating movement of the sensory hairs. As all the vestibular epithelia except the macula lagenae face more or less upwards and backwards, the constant hydrostatic pressure of the endolymph will produce a tonus reaction directed upwards and backwards, counteracting the influence of gravity and the tendency to topple by advancing. This reaction may be increased by injection of pilocarpine salicylate and quinine (Dederding [2 3]), producing an increased endolabyrinthine secretion (fig. 6). The opposite reaction, the loss of tonus,

may be seen by temporary setting the labyrinth out of function by instillation of chloroform oil in the external meatus, unilaterally (fig. 7) or bilaterally (fig. 8).

These observations and considerations were published 1924-1927 (S.H. Mygind [1 2 3-4]), not without several admittedly false conclusions. 1927 the experiments of Versteegh disproved the whole system of Magnus and de Kleyn. To my opinion their many very fine experiments in so far have kept their importance, as any acceptable labyrinthine theory must be in accordance with their experimental facts, a proof hithertoe disregarded by all other aothors.

In the meeting of the collegium in 1948 (S.H. Mygind[4]) I sought to form a consequent synthesis of the then existing knowledge of the vestibular labyrinth and my own ideas, and maintained that the vestibular function depends upon the size dominance, spatial position or »fronts and curvature of the epithelium in question. As in the saccular macula as well dominance as curvature are very weak, and as all eye reactions are rotations, vestibular saccular reactions become very difficult to observe. Therefore the old belief that the saccular macula had no static function. I made an alteration from my former view which considered the posterior part of the saccular macula as dominant. Certain experiments showed that the anterior part must be the dominant. Before this, but without my knowledge, Werner[1] had made some very minute histological measurements of the saccular macula, showing the anterior part of the epithelium as slightly but constantly higher than the posterior. Later fine experiments of Jongkees have demonstrated a saccular function, just as theoretically foreseen. The experiments by Magnus and de Kleyn were found in accordance with the new theories. The only fault of the system presented, was, as far as I can see that the pressure stimulation was thought as depending upon piezo-electrical processes. The correspondance with the motor-reactions by monocular prawns (Alverdes) unilabyrinthine vertebrates and cray fishes (Kuhn) and certain motor-correspondances of the feeling of touch were further described and depicted (Mygind[4]).

In a series of lectures on aural medicine (S.H. Mygind[8] 1952) I took up the whole problem again, largely widening the knowledge of the law of dominance by histological material. *)

Then came (1953) the next decisive step forward with the discovery of hyaluronic acid in the labyrinth by Vilstrup [1 4] and the following studies by J.A. Christiansen and their co-workers. The hyaluronic acid forms giant molecules, very slender with a length of about 0,5 my When bended they emit electric potentials, which give rise to stimulation. J.A. Christiansen believed the molecules to be sitting on the hearing hairs, but here is hardly room enough. I have therefore (S.H. Mygind[12]) proposed a modification of this theory according to which hyaluronic molecules form bridges over the interval between the hair cells and their appertaining supporting cells in such a way that the molecules become curbed by the slightest dislocation between these two forms of cells, resulting into an electric firing of sti-

*) The polarity of the kinocilium in relation to the stereocilia, demonstrated by electronic microscopy by Wersäll and his coworkers (Loewenstein, Osborne and Flock) does not seem to have anything directly to do with dominance, although the two phenomena may follow each other as in the crests, but only partly in the utricle. In the saccule they do not correspond. In the lagena the polarity is reversed from the one neighbouring cell to the other.

mulating outputs. As far as may be judged from the histological material from the lateral organ (Flock and Wersäll) and the vestibular and acoustic epithelia (Engström and others) structure and dimensions do not speak against the theory Such stimulating dislocations seem to be just of the molecular dimensions required by the calculations of de Vries [2 3] (See fig. 9)

In the cochlea a dislocation between sensory cell and supporting (Deiters') cell is only possible by oppositely contemporaneous influences and therefore only over a restricted area. (For details see S.H. Mygind[11] fig. 15). The same mechanism also hinders a producing of a tonus in the cochlea. v Bekesy s ingenious experiment of exposing the organ of Corti of a living guinea-pig to various frequencies, while observing the movements produced was found in full correspondance with our hearing theory In the same paper the fish experiments of v Holst and co-workers were shown to be only a tautological pseudo-proof. Also the theory of the meritious Breuer of gliding otoliths, equipped with special furrows, was unveiled as a histological misunderstanding. It was further demonstrated that the cochlear microphonics do not represent the nervous stimulation, but are caused by pure mechanical processes in the organ of Corti and the tectorial membrane spreading widely by low frequencies even over the whole length of the cochlea.

The vestibular tonus reaction with its upward and backward direction, against the influence of gravity and the tendency to topple by advancing, is caused by the hydrostatic endolymphatic pressure acting on the vestibular epithelia, all, except the macula lagenae facing more or less upwards and backwards. This is reflected in the differences of the postrotatory vertical nystagmus, the upward deviation or the downward nystagmus being double as strong as the oppositely directed. Some apparent discords in the rotating experiments of Arrelano are explained by a nystagmus reaction always being stronger when it coincides with the tonus reaction being syntonic than when it goes against it, being antitonic The tonus reaction is as already mentioned, clearly demonstrated in the experiments of Dederding. *)

That a gliding of the otoliths under certain conditions, however is possible, is demonstrated in the X ray experiments of the Vries[1] and of Vilstrup[3] But an otolith or a cupola cannot glide without exercising at the same time a pressure at the one and a traction at the opposite part of the neuroepithelium. Particularly the heavy fish otoliths are hampered in their gliding movements by a specially developed system of strong strings(Werner[1]). Such are also met with at the saccular macula of the frogs. **)

) As the paper is going into print, I quite accidentally discover that Ledoux[2] already in 1948 observed, in frogs, that such reactions from a certain crest increased and decreased according to the position of the head. When the ampulla was upward, the reactions increased, while they decreased when the ampulla was downward. In the following discussion Holzinger suggested wisely that such difference might be due to a hydrostatic reaction.

) Investigations of Vilstrup seem, however to show that such string are artefacts, caused by a shrinkage of the very large gelatinoous masses enveloping the otoliths. Ulrich also made very minute stimulation experiments by pressing a hair at different points of these masses. The results are not quite according to expectation, though better than his predecessors Kubo and Maxwell, the gelatinous masses, probably also existing in higher vertebrates in a more limited degree, may perhaps have a retarding influence on the otolith reactions which at least in certain cases seem to require some time for reaching their maximum.

We should at this place perhaps preliminary remark that an increase of this physiological tonus reaction may be noticed as an everyday observation in normally hearing individuals who when they are lying on their back with their head hanging downwards, as by suction-treatment for sinusitis, very often complain of giddiness and at the same time, behind the spectacles of Bartels, present a distinct spontaneous vertical nystagmus. The downward direction of this proves that it is released as an increased tonus reaction on account of the increased endolymphatic hydrostatic pressure and not as physiological otolith reaction. In the last case namely the course would have been just the opposite. These observations fit with the so-called rocking experiments of Falbe Hansen[2] carried out at the suggestion of Krogh and controlled by him in his laboratory In these a normal individual, with the head hanging, regularly shows a moderate hard bearing with bass deafness just as in our Meniere cases. Observations and experiments thus correspond with our pressure theory At the same time the intraocular pressure became increased.

The tonus reaction gives of course rise to a continual fire of potentials from crests and maculae (Loewenstein and Sand[1 2] Ledoux, Loewenstein and Roberts).

It is our intention to see how these views, principally based on pressure, dominance, spatial position or front, curvature and size of the individual static epithelia, are able to explain the variations found in a series of vertebrates in accordance with their various ways of living and particularly of locomotion.

The principal material for our investigations has been the work of Retzius. Notwithstanding its more than 80 years of age its value remains untouched, as far as our purpose is concerned. It embraces 91 different vertebrate species. All the illustrations have been done by one man, Retzius himself. In many cases I have found details in them, not mentioned in the text, but which I have been able to verify myself.

My dimensional statements are admittedly approximative. We particularly should like to point out the difficulty in determining the dimensions of the macula utriculi and its otolith. The macula often extends mostly in transverse direction and presents itself seen more or less from the edge, i.e. comparatively too small. The ciphers given in parenthesis refer to the relative size of the maculae of the utriculus (u.) sacculus (s.) and lagena (1).

In the following I have used the old-fashioned order of Retzius (R) as being my main source, and have put informations from other authors in between.

Besides I have personally examined more than 70 vertebrate species histologically many in several specimens from the collections at the Anatomical Institute of Groningen (G), the Embryol. Institute of Lund (L) and the Comparative Anat. Institute of Copenhagen (Cp) and added as extensive literary studies as possible. The total material therefore by far surpasses that of any prior publication on this subject.

I. Cyclostomata.

The hag-fish (Myxine) is generally considered as a degenerated form with only one macula communis and one (vertical) canal with two crests (fig 10) It should, however be remarked, although not mentioned in the text, that the

lower part of the macula is depicted with a somewhat steeper fall of its lower border showing this part, according to rule, to be dominant with its nerve coming from above. The macula shows typical tendency to divide in two opposite halves. The crests (fig. 11) of the only existing vertical canal show a decided dominance of the side supplied from the peripherical part of the nerve

Regarding the lamprey (Petromyzon) there is a more modern description by de Burlet and Versteegh (fig. 12). According to their scheme the relations of size between the macula sacculi, the macula neglecta, macula lagenae and macula utriculi should be something like 1 2-4-8 a relation not met with in any other vertebrate. As in the embryonal state of higher vertebrates, there is no sharp division between the individual maculae. De Burlet and Versteegh describe how the extensive macula utriculi is situated horizontally while the extremely small macula sacculi and the large macula lagenae have nearly vertical positions. The lamprey has only two (vertical) semicircular canals, embracing two ciliated endolymphatic sacs. In the large ampullae the crests are furnished with a septum cruciatum (fig. 14) The function of the ciliated sacs has been explained by me (Mygind[5]) as presenting a sort of gyroscope mechanism. The constant circulating endolymphatic currents, set up by the cilia in the sacs (fig. 13-14) will, by changing of position in the horizontal plane continue their direction, get free, and as may be seen from fig. 15 be directed out into the ampullae with opposite directions in each of the halves, twisting both cupulae the same way round. My formerly published illustrations have, unfortunately become faulty but the text is allright.

The ostracodermes living for ca. 300 millions of years ago, now found as fossils, particularly at Spitzbergen, and thouroughly examined by Stensioe, were cyclostomes (fig. 16). They had also only two semicircular canals, but no endolymphatic ciliated sacs. They lived burried in the mud with their body and their large heavily armoured head free A little over the lower border of their head and again on its top they had an organ which will be discussed later (page 13). They had an immoveable parietal eye which probably furnished them with a vertical orientation by the light coming diffusely down through the water The organ presents a distinct anterior dominance. As the parietal eye is immoveable, without muscles, it cannot have worked together with the ordinary eyes by positional changing and thus cannot have been an organ of sight (Mygind[7]).

II Fishes, including Plagiostomes (Sharks and Rays).

As water animals all these species have no orientating contact with the ground. The extraordinary demands have resulted into relatively very increased dimensions of their labyrinth. The semicircular canals are particularly developed in the flying-fish (fig. 26) The septum cruciatum of the ampullae of the lamprey has disappeared as being superfluous after the developing of the horizontal semicircular canals. In the dog-fish (Acanthias) Vilstrup found a distinct difference between the external crest on the one and the vertical crests on the other hand. In the first the side of the sensory epithelium facing the utricle (fig. 17) is somewhat more curbed, perhaps some-

what higher and certainly shorter In the vertical canals (fig. 18) the opposite relation was found. In my opinion the shorter parts represent the dominant ones.

When we regard the 45 different species of fishes represented by Retzius it is immediately seen that the saccular macula, with exception of the bow-fin (Amia, fig. 19) (u. 1 1/2, s.1, L2) and to this we may add the Calamoichthys (u. 1 1/2 s. 3 L5) described by Greve (fig. 30) always is the largest. In the sturgeon (Acipenser fig. 20 u.1, s.3 L2) the macula sacculi is only somewhat larger than the mac. lagenae

It is further seen that large maculae sacculi and great speed correspond Perch (Perca. u.1, s.20 L1) pike-perch (Lucioperca Sandra, u.1, s. 20 L1) mackerel (Scomber u.2, s.10 L2) weever (Trachinus Draco u.1, s.25 L1), goby (Gobius, u.2, s.30 L1) dragonet (Callionymos Lyra) u.1, s.12 L2) wolf fish (Anarrhichas Lupus, u.2, s. 20 L1), cod (Gadus Morrhua, u.2, s. 15 L1) lesser fork-beard (Raniceps Ranina, u.2 s. 20 L2) sole (Solea, u.1, s.20 L2) salmon (fig. 22) (Salmo u.2, s.10, L2), herring (Clupea, u.1, s.5 L2) white fish (Corregones, u.1, s.8 L1) pike (fig. 23) (Esox lucius fig. 23 u.1, m.10 L1) eal (Anguilla vulgaris, u.1, s.10 L1) dog-fish shark (fig. 24) (Acanthias vulgaris, u.s. s.10 L1).

In all these cases the maculae utriculi and lagenae remain small, the macula sacculi surpassing them 5-20 times in size. One of the most excessive relations is found in a pelagian fast swimmer the garfish (Belone vulgaris, u.1, s.20, L1). But comparatively considerable size may also be found in some bottom fish, particularly such of rapacious habits and therefore equally fast swimming (Gobius, Anarrhichas).

Most bottom-fish are, however slowly moving, and have distinctly smaller saccular maculae Gar-pike (fig. 21), Lepidosteus (u.3 s.5 L1) mullet (Mullus barbat. u.1, s.7 L1) Pagellus cemtrodontus (u.2, s.8, s.8, L2 1/2), John Dory (Zeus faber., u.1 1/2 s.4 L1) angler (Lophius piscatorius, u.1, s.9 L1), grey gurnard (Trigla gurnardus, u.1, s.5 L1) fifteen-spined stickleback (Gasterosteus Spinachia, u.3 s.5 L1) fig. 25) wrasse (Labrus mixtus, u.3 s.10, L2), trunkfish (Ostracion cornutus, u.1, s.3 L1) sea-horse (Hippocampus, u.1 1/2 s.2 L1/2) needle-fish (Siphonostoma typhle u.1 1/2 s.2 L1/2). Some root in the mud and feed on worms and other small organisms, as the sturgeon and the bow-fin or mud-fish, or on larger slowly moving prey as mollusca, crabs. In such fish the saccular but not the two other maculae, are reduced.

In the following fish the whole inferior part of the labyrinth with all 3 otolith organs are very much reduced.

In the flying-fish (fig. 26) (Exochoetus volitans) all maculae together with their otoliths and nerves are very much reduced, while, as said before, the semicircular canals are particularly large. All this is in strictest accordance with the moving habits of the fish. During the flight over the water the possession of strong otolith righting reflexes would impede the flight by making the large brest-fins brake the projectile-like movement and also by carrying the body back to its normal position in relation to the horizontal plane. In the porcupine-fish (Tetrodon Mappa) the otolith organs are also very mush reduced, particularly those of the sacculus and the lagena (fig. 28). The animal likes to inflate itself with the belly turned upwards and float, swinging up and down on the top of the waves. It has consequently no use for righting reflexes. Only the comparatively large upward facing utricular maculae may furnish it

with a special vertical orientation. In this way therefore it may remind of the air type later to be met with. In the lumpsucker (Cyclopterus Lumpus) all 3 otolith organs are very much reduced (fig. 27) The fish sits fastened with its suckers immoveable to a stone or a piece of floating timber without any use for righting reflexes.

The mac.utriculi and lagenae are in most fishes of the same size. A difference is, however found in favour of the utricular macula in the following species: Lepidosteus (fig. 21), Gobius, Callionymus, Anarrhicias, Gasterosteus, Labrus, Tetrodon Siphonostoma (needle-fish), Hippocampus (sea-horse) Anguilla (eal)

In several of these cases the preponderance of the upwards facing utricular macula does not seem to be accidental. The ancients Lepidosteus has often to seek upwards for its air respiration The utricular tonus reaction will favour this ascending movement The same is perhaps the case in the eal, where it may help the fish to cross by land to another water and it is remarkable that the only 3 nest-builders in the material belong to this group (Gobius, Gasterosteus, Labrus) The explanation may be that the complicated positions and movements required in their special work embracing i.a. side-positions and vertical changes, are favoured by the comparatively large utricular maculae The utricular predominance in the Tetradon has been discussed above In the Siphonostoma and the Hippocampus the comparatively large utricular maculae correspond to the vertical horse-like carriage of these fishes. No explanation for the dimensional difference between the two minor maculae is to be found in Anarrhichias nor in the Callionymus.

The maculae lagenae are on the other hand larger than the maculae utriculi in Acipenser Amia, Calamoichthys, Plagellus, Solea and Clupea. The difference is, however only pronounced in the first three species, where as said above the macula lagenae reaches or even surpasses the macula sacculi in size All these fishes rood at the bottom or live in muddy water In the Amia also the maculae utriculi are comparatively large, probably because this, also ancients fish is an air-breather

For the small difference mentioned in the Plagellus and in the Clupea I found no explanation. For the Solea it might be suggested that it lives burried in the bot tom, watching for prey But other flat fish as the rays, using the same trick, show as we later shall see, rather a preponderance of the utricular maculae

The ostariophysarian fishes as sheat fish (fig. 29) and carps have a connection (The Weberian ossicles) between their swimming bladder and their labyrinth. And the forepart of the macula sacculi is transformed into a hearing epithelium (de Burlet[1]), a device that was unknown to Retzius, (just as the probable hearing capacity in the herring of a part of the maculae utriculi (Tracy). But what, however can be seen is that only about one half of the macula sacculi is left as static epithelium And the whole saccular macula is only of about the same lenght as the utricular or the lagenar maculae, while at the same time its vertical dimension is considerably lower The usual very conspicous superiority of this macula has thus changed to a distinct inferiority reducing its static part to about 1/4 of each of the other two maculae, which are of about equal size.

Yone Z. Nakagava has by altering the air pressure in an airtight aquarium exposed ostariophysarian fishes to an increased air pressure which immediately made them ascend while a descreasing pressure made them seek the bottom and at the

same time spit air This is in due accordance with our tonus theory But leaches and cat-fish did not react or showed inverse reaction. Such fishes are bottom dwellers (unknown tonus regulating reflexes ?).

The considerable size of the utricular and lagenar maculae in all these fishes is obviously caused by the change of function in the anterior half of the saccular macula. The greater part of its static activity must have been transferred to the two other otolith organs. There is, as said above no principal difference in the perception nor in the reactions of the different otolith organs. It only depends on their front, curvature, dominance and size. It is therefore easy to explain, how the vertical outwards and somewhat forwards and backwards looking parts of the lagenar macula necessary will react on rectilinear horizontal accelerations and in this way substitute the usual function of the macula sacculi. The reduced (partial) vertical function of the macula sacculi is probably taken over by the enlarged macula utriculi.

Particularly in the Malapterurus (fig. 32) the electric sheat-fish, also an ostariophysarian, the utricular and the lagenar maculae are of a quite extraordinary size for a fish. It should be noted that there are several other electric fishes belonging to this group.

It was originally my idea that the macula lagenae had something with digging and bottom seeking habits to do, as we in higher vertebrate species with such way of living have a more or less downward facing macula lagenae. In the 5 osseus fishes: Esox Perca, Gadus, Cottus (sea-scorpion) and Scardinius the macula is formed as a flat cup situated on the inner wall of the lagena, facing outwards and sometimes also more or less forwards or backwards or with its upper border even somewhat downwards. The otolith is hanging at the side of its macula (Werner[1]).

This shows us that the macula lagenae should be expected principally to react to horizontal rectilinear accelerations, coming transversely This means that its function should be very much like that of the macula sacculi from which it has developed.

Neither in the plagiostomes we find any indications for the lagena having anything to do with a downwards directed tonus or with a special disposition for digging habits, as the macula lagenae in most, but not all species is rather poorly developed, also in the rays with their typical digging habits. According to Vilstrup[3] the mac. lagenae faces outwards in sharks, but upwards in rays. This is in accordance with the directions to be expected of mechanical vibrations from the surroundings.

A more intimate study in the plagiostomes, based mainly on the work of Retzius and the also very solid work of Werner[2] (1950) give some important informations on facts observed, but not understood before, relating to the vestibular anatomy and the way of living of these animals.

The sharks rush at their prey and catch it with their sharp teeth. In this manoeuvre they make use of a sudden arresting, producing, as in all vertebrates, a pressure of the otolith on the dominant anterior border of the maculae utriculi. Vilstrup[2] has a histological picture of this utricular dominance in the Acanthias, corresponding to the findings in Calamoichthys by Greve (fig. 31). It is, according to the direction of the nerve, always particularly well developed in sharks, as in the sea-cat (Scyllium Canicula, fig. 33) and the angel-fish (Squatina Angelus, fig. 34) and further in the Pristiurus melanostomus and perhaps also in the Galeus canimus (Werner[2]). To this may be added the Pristiophorus Japonicus (Iseistöger[1]). This special possibility of

is lagenae in lung-fishes is difficult to understand Is it to avoid vibrational disturbances from the exterior that might disturb the encapsulated »sleeping» fish with its extremely reduced metabolism ?

On the other hand we have as said, met three fishes with remarkably small maculae sacculi and large maculae lagenae the maculae utriculi being of the usual small size in fishes. In Amia (fig. 19) and Calamoichthys the macula lagenae is very large, larger than the macula sacculi. In the Calamoichthys the histological reconstruction by Greve (fig. 30) shows a practically vertically standing outward facing positions of both these maculae The sturgeon (fig. 20) roots, as said the muddy bottom for small organisms. The Amia is also called the mud-fish and the Calamoichthys lives in muddy rivers. One cannot help contemplating whether a reduced possibility for sight has been compensated for by a development of sensitivity for mechanical vibrations, just as the same deficiency in muddy waters have been made good for in other fishes by electrical communication (Lissmann). Such vibrations will principally progress horizontally

The macula lagenae has developed from the macula sacculi with which it in the early stages often is seen to coalesce or not to be recognized as independent organ. In its position it often seems to follow the macula sacculi. The circumstance however that it, on a whole does not follow the dimensional variations of the macula sacculi, neither when this is particularly large or small, even surpassing sometimes the macula sacculi in size corroborates the suspicion of a quite special function belonging to this organ.

When I in 1926 founded the rule of dominance I believed the posterior part of the saccular macula to be the dominant apparently being the most peripheral. Some years later I met Werner in Hamburg. He was very sceptical and doubted the possibility of histological verifying real differences in height of the epithelia. Afterwards, in order to explain certain experimental facts, I as above said recognized that it was the anterior outer and in this way more peripheral part of the saccular macula, that must the dominant (1948 Mygind[2]) In the meantime Werner[1] had made investigations with some very careful measurements, which (before me) proved the correctness of the final rule measurements which only recently have come to my knowledge. In the macula sacculi the anterior part was found a few my higher that the posterior part reaching in the scorpion-fish (Cuttus scorpio) to 39 my against 36 my In the mac. utriculi the difference between the dominant anterior and lateral part (Rampa) and the subordinate central inner and posterior part (Cotillus) was always much greater reaching f inst. in the tench (Tinca) from 60 my to 15 my Such differences of height Werner never observed in the lagenar macula.

Werner strange to say gives no information of the dominance relation in his extensive work on the plagiostomes. In an embryo of a dog-fish (fig. 41) I found, as expected the lower part at the saccular macula dominant Vilstrup[3] found the anterior and exterior part of the mac. utriculi in the Acanthias, as said as the highest as is seen, but not described in the photos of Greve of Calamoichthys (fig. 31).

Werner[1] has further given a very thorough description of the otoliths, the otolith membranes and the maculae in osseous fish. As a rule a part often a considerable part of the macula is found perfectly naked without any connection with its membrane nor with its otolith. Vilstrup[3] basing himself on his histological expe-

rience in Acanthias, thinks however that also in osseous fishes, such areas have been covered by gelatineous and fibrous tissues, later destroyed by the preparation.

In all the osseus fishes, treated by Werner[1] he found the comparatively enormous bony otoliths riding with their relatively small central part and resting on their maculae with the otolith membrane interposed. This may take a shape more or less reminding of that of a semicircular canal cupola with a striated, tubular structure, as seen in the lagenae of the cod-fish (fig. 42). Maybe there is even a central space filled with endolymph (?) between the otolith and its macula. The otoliths have enormous »wings« the weight of which will make the otoliths with a lever effect tip over around their central connection with their macula, exposing this to a strong pressure on its lower and to a strong traction on its upper part. Very strong fibers arising from the marginal epithelium of the maculae and sometimes also more peripherically fasten the otoliths and counteract a gliding, but not a tipping over. The whole arrangement is distinctly more pronounced in the principally vertically standing saccular and lagenar maculae than in the principally horizontally situated utricular macula (fig. 43). As far as I can see, the result must be the aquisition of a greatly increased power to resists a capsizing, a reaction otherwise principally depending on the macula utriculi. This is, however in fishes, as in most water animals, only small. And perhaps there may even result a faculty of reacting to curved accelerations.

The plagiostomes, the lung-fishes and other ancient forms of fishes have no otoliths, but a rough powder of otoconia embedded in their otolith membranes. The plagiostomes have also particles of sand, brought in via their open endolymphatic duct. Here the above arrangements are of course absent. The risk of capsizing of the osseous fishes, generally having comparatively small transverse and large vertical dimensions, becomes of course negligeable in the flat rays. But also the sharks have rather broad bodies. The risk is further counteracted by the often excessive development of their dorsal fin. In lung-fishes a capsizing is counteracted by the size of their large utricular maculae.

And here we may add some remarks prior to as well the real fishes as the plagiostomes. The Myxine lives dug down into the bottom. Neither its worm-like living here, nor its crawling around in dead fishes, feeding on their entrails, make a special body position necessary Neither does its vestibular organs indicate any features of a special static orientation. In the Petromyzon we meet the only water vertebrate with on the one side a quite minimal macula sacculi and on the other side and overwhelmingly large macula utriculi. The macula lagenae is also extraordinarily large, only surpassed by the macula utriculi. It is, as said, vertically standing and outward facing and may have taken over from the macula sacculi the function of reacting to horizontal transverse accelerations, as suggested in the ostariophysarians. Such movements may perhaps come into play when the host tries to free itself from its parasite. The very reduced size of the saccular macula, seems to suggest a very poor swimmer The dimension and the position of the lagenar macula might also indicate a sensibility for mechanical vibrations, in certain situations presumable the only influence from the outside able to reach the animal. As to the for a water animal extraordinarily large macula utriculi, it may be, that the absence of the horizontal canals with their crests facing upwards and somewhat backwards may cause a corresponding lack

in the usual upward-backward directed tonus reaction demanding a compensation from the utricular macula. A strong upward directed motor tonus may perhaps also be of importance for the animal in seeking for its prey and advancing in its interior

Regarding all the fish-like species as whole we will see that they with a few exceptions, with their comparatively large saccular and small utricular maculae answer to the type of water animals, which we earlier have sat up founding on our principles of pressure and dominance. The mainly outward and backward facing, nearly vertically standing and downwards always dominant macula sacculi has a size and a facing corresponding to the horizontal, transverse as well as longitudinal, rectilinear accelerations to which the animal is mainly exposed from the surrounding water The influence of gravity is counterbalenced by the buoyancy force of the water The macula utriculi is therefore only small. Neither is there in fish-like animals the tendency to topple over by arresting of a forward movement. The outer part of this macula is, as in all vertebrates, always dominant.

III Amphibia.

All amphibians are particularly when compared to the following group rather stiff-necked according to their possesion of two-nape knots. The cristae of their semicircular canals are smooth without any septum cruciatum just as in the stiff neck ed fishes. The macula sacculi is always dominant in its lower part.

A. Urodeles.

This first group is by Retzius represented by 10 species Proteus anguinus (u.l, s.4 l. 1 1/2, small gills), Siren lacertinus (u.l s.4 l. 2, all rather small, no hind legs), Menobranchus maculatus (u.l, 3 5 l.l, gills), Amphiuma means (u. l, s.5 l.l, gills), axolotl (Siredon mexicanus, u.l, s.4 l.3 all very small) crested newt (Triton cristatus, u.l, s.2 l. 1 1/2 all rather small), (fig. 44), Pleurodeles Walthi (u.l, s.3 l.l all rather small), hellbender (Menopoma alleghan, u.l, s.20 l.l), spotted newt (Salamandra maculosa, u.l, s.l 1/2), Crecilia annulata (u.l, s.l, l.l, wormlike, without legs).

They all show relations very much resembling those of the fishes, especially the slow moving forms of these. Only in one, the salamander like, gill-less Menopoma an overwhelming development of the saccular maculae is found with at the same time only small utricular and lagenar maculae. In the other forms the saccular predominance, though existing, is, comparatively limited The utricular and lagenar maculae are about of the same size. In none of the species represented the macula lagenae reaches quite the macula sacculi in size

All urodeles have low and slender legs. Their toes are without claws nor swimming webs. Legs may even be wanting. In the water they swim adroitely as fishes by means of their tail. On the ground they move clumsily They do not dig, but may hide themselves in crevices, between leaves etc.

In preparates of a newt (Triton vulg.) which I have had opportunity to examine (Cph.) I found the same distinct dominance of the utricular macula as in other verte-

brates and the same position and lower dominance of the macula sacculi as met with in fishes and sharks. The macula lagenae faces outwards and also distinctly upwards (also seen in illustrations of Harrison). The same I found in a preparate of Ichthyophis (L.). Mechanical vibrations in the shallow water will hit horizontally and probably often somewhat obliquelly from the entouring brinks above.

B Anurea.

The limbs are here much stronger particularly the hind-legs, which have developed into saltatory legs. They are often furnished with swimming web, but have no claws. Most anures are able to dive and dig. 5 species are represented by Retzius: Common toad (fig. 45) Bufo vulgaris (u.1 s.2, L4) Tree-frog (Hyla aborea with sucking discs on its toes, u.2, s.3 1/2 L1.), Midwife-toad, (Alytes obstetricans, u.3 s.4 L1), Spate-footed toad (Pelobates fuscus, u.1 s.3 L3), Common water-frog (fig. 46), (Rana esculenta, u.1, s-.3 L2).

That the special »fish-macula« the macula sacculi, is only of half the size in the common toad as in the water frog, may hardly be accidental, the first one being principally a land the other a water animal. The large saccular otolith consisting of chalky masses is fixed by a membrane with marginal fibres (shrinkage of gelatineous masses ?).

In the Pipa americana (fig. 47) the maculae lagenae are seen leaning outwards and downwards about 20° from the vertical (L). The same position is seen in casual or partial illustrations by Weston and by de Burlet[1] The downward facing position of the lagenar macula fits, according to our theory with a downward directed motor reaction, showing itself by the digging and diving habits of the anurea. The possibility of a sensivity to *mechanical vibrations coming from the side and from below* may however also be taken into consideration. We all know how apt the frogs are to jump into the water how cautiously and well covered we are nearing ourselves their bank.

McNally and Mac Naughton have cut the lagenar nerve on the one side in a frog. This resulted in the animal lifting the operated side upwards, i.e. in full correspondance to the anatomical findings and our theory of a hydrostatic pressure stimulation of the vestibular labyrinth. That experimental destruction of the lagena in fishes has on the other hand been without result (Werner v Frisch and Stetter, Loewenstein) cannot wonder McNally and Mac Naughton on the other hand found that a frog bereft its utricular and saccular maculae, but still possessing its lagenar maculae intact had still its righting reflex preserved.

The macula neglecta will be treated in a following separate publication.

IV Reptiles.

All reptiles have only one nape-knot. This allows an extreme mobility of their neck. They are consequently particularly exposed to curved accelerations. This is

probably the reason for their having a septum cruciatum in each of their vertical ampullae, just as before met with in the lamprey All reptiles have claws, they »grip» and cling more or less to the ground. Most of them dig.

A. Tortoises.

4 species are represented by Retzius: europ pond-tortoise (Emys lutraria, u.l, s.5 l.2) river-tortoise, (Trionyx subplanus, u.l, s.5 l.2) sea-tortoise (Chelodine, u.l, s.5 l.2) alligator tortoise (Chelydra serpentina, fig. 48 u l, s.4 l.2). The macula lagenae has thus the double size of the mac.utriculi and nearly half the size of the mac. sacculi. In the Chelydra the Chelodine and the Trionyx Retzius expressly describes the cochlea with the lagena as inwards directed As the macula always expands particularly medially this means that it is facing downwards with its main part.

All tortoises dig. In the land-tortoises all toes are united forming a club-foot The other tortoises have their toes separated and furnished with very strong claws. Most tortoises swim.

Their small maculae utriculi and large maculae sacculi indicate them still as water animals, only the increasing size of the maculae lagenae makes a difference. In the Testudo graeca I found a modest size of the maculae utriculi with pronounced dominance of the outer part (Cph) In 3 different preparates (L.) of the Chelydra I found the usual inferior dominance of the macula sacculi, the lower part of which is sloping more outwards than the nearly vertical main part. This is at the same time somewhat increasing gradually in epithelial height upwards, leaving a »neutral» part between it and the lower distincly dominant part. There seems also to be an indication of such »neutral» part in foreward-backward direction, the anterior part being as usually the dominant. A corresponding differentiation I did not remark in the Emys or in the Chelodine. In the Testudo graeca (Cph) I have recently sought it in vain. The Chelydra has a relatively small carapace and is very mobile. In the picture of Retzius the distribution of the saccular nerve shows a corresponding difference between the Chelydra on the one side and Emys, Trionyx and Chelodine on the other side.

The lagena forms a sac narrow from side to side with its upper border sloping downwards and forewards (Miller). The macula is situated at the top expanding more or less on both sides (fig. 55 D). The type is more or less repeated also in the following reptiles. As a whole the macula is downward facing.

B Serpents.

Only 5 are represented by Retzius. horned viper (Vipera Rhinoceros, u.l 1/2, s.2 l.4) rattle-snake (Crotalus horridus, u l, s.l, l.3), Phyton Zebae (u.l, s.3 l.4), Zamanis hippocrepis, (u.l, s.l, l.4) Coelopeltus Lacerta (u.l, s.l, l.5).

In all these the macula lagenae has become the dominating otolith organ. If we conceive the lagenar function as consisting in an incentive to a downward moving, it will be seen that this is exactly what the serpents are needing in order to advance, pressing their abdominal scales against the ground. In the Vipera (fig. 49), the Crota-

hus and the Zamenis the somewhat reduced saccular nerve still runs distinctly downwards as in all vertebrates hithertoe described. In the Phyton (fig. 50), however nearly one half of the fibres run upwards, a trend which we will find developing further in the following group where it will be explained. The macula utriculi is always of modest size.

And here we find the position of the macula lagenae which we have sought for In the Vipera no special description is given. But in all the other four cases it is ex presely described by Retzius how the lagena is directed not only downwards, but with its lower part also inwards, i.e. the macula must be principally downwards fac ing.

Miller describes the serpent lagenae (in Pithuophis catenifer) as having ordinary reptile shape with the mac. lagenae lining the anterior border and the inner side.

C Saurians.

12 species are represented by Retzius: Phrynosoma cornuta (u.l, s.l, l.5), chamaeleon (Chamaleo vulgaris, u.l, s.l, l.5) glass-snake (Pseudopus pallasi, u.l, s.2, l.4), slow-worm (Anguis fragilis, u.2 s.l, l.2 1/2 3). Iguana tuberculata (u.2 s. 1 1/2, l.4), gecko (Platydactylus, u.l, s.l, l.4) green lizard (Lacerta viridus, u.2, s.l, l.4) eyed lizard (Lacerta ocellata, u.? s.l., l.4) monitor (Psammosaurus casp., u.? s.l, l.4) (Plestiodon Aldrovendi, u.l, s.l, l.3), scink (Egernia Cunninghami, u.? s.2 l.3), Sphenodon (Hatteria) punctata, u.? s.l, l.3

In the Anguis fragilis, the slow worm all 3 maculae are (fig. 52) of equal size but in all the other species represented the macula lagenae is by far the largest, surpassing the macula sacculi in many cases by 4 to 5 times (Chamaeleo (fig. 51). Platydactylus, Lacerta (fig. 54)). All the saurians cling more or less to the ground, hooking on to it with their well developed slender grasping claws; many dig and hide in holes. The specially developed prehensive feet of the Chamaeleo are better adapted for clinging to a branch than to make a real walk along it. The same is the case in the Iguana.

In the saurians a very interesting development takes place. In real water vertebrates a sideward push will only tend to dislocate the animal towards the side. First when supporting legs develop a possibility for tipping round over the legs in this situation begins to start. But as long as the limbs are principally tools of support, with the feet directed more outwards than forewards, the risk is minimal. When the limbs, however become principally tools of walk and therefore longer and not so much as before directed outwards, but forewards, the equilibrial situation alters radically and the animal comes in danger of tipping over its feet. This altered situation is reflected in the vestibular structure. In all vertebrates below the saurians the saccular nerve is directed outwards and downwards. The lower part of the macula consequently always in dominant as histologically demonstrable as mentioned before and f.inst. visible in the Protopterus (lung-fish), fig. 39 The same I found in the Triton and in the tortoises.

In the real saurians (except in the slow-worm) the saccular nerve points always upwards. The upper part of the epithelium thus becomes the dominant. By this

change of dominance a sideward push will no longer elicit a straight oppositely directed motor reaction, but, owing to the upper dominance, an upward curved reaction, against the push, counteracting the tendency to tip round over the feet.

This changed upward direction of the saccular nerve is seen in all saurians represented, except, as said in the slow-worm, that moves like a serpent. In its near relative the glass-snake however the upward directed change of the saccular nerve has already taken place. The size of the macula is in all saurians only modest.

On the other hand, in the Python from the former group a good part, nearly 1/2 of the saccular nerve has already become directed upwards, probably because this serpent is living in trees, where the demand for equilibrium are otherwise than in the terrestrial forms (fig. 50).

The lagena in the saurians is of the type already described in the lower reptiles (fig. 55 C) Its sac form with a generally downwards sloping anterior border is clearly seen in the 18 different species depicted by Miller in drawings and stereophotos too small to be reproduced Of the Crotaphyllus wizlizeni, a lizzard species, there is, however a picture sufficiently large for reproduction (fig. 53) giving a general impression of the saurian lagena. In the Diploglossus lessonae a near relative of the slow-worm, and as this probably limb-less, the mac. lagenae is according to the picture and the description mainly limited to the downward facing inside of the lagenae just as in the serpents. The macula is in the other cases generally described as sitting at the top and expanding particularly at the medial side. As it is seen through the non-opened wall and as the otoconia have been dissolved by injection of a vinegar solution, the expanding of the macula and its nerved is rather indistinct. The transverse diameter being much smaller than the longitudinal and vertical, the organ, consequently on frontal sections presents itself as a tube or tunnel, broadening somewhat downwards. And if the section has hit only a little too far posteriorly the top of the macula will not be seen, but only the part of the macula expanding on the medial and the lateral side This is the case in preparates of the Iguana (G.) (fig. 55 C). In a horizontal section of the lacerta (fig. 59) the macula in its upper part is seen forming a ring, open only at its medial side, where it leaves room for the downwards increasing organ. At the bottom the lagena is always nude, except in the gecko, where it is covered by the macula, as seen in fig. 55A, a scetch I made from the collection of Groningen, probably the same preparate as depicted by Weston[2] (fig. 7) only the very lowest part of the photo has here been cut off. This very important state has not been mentioned in the text. The same photo is apparently the same, somewhat indistinctly reproduced, met with in the text-book of de Burlet. It has also escaped the notice of Hamilton and also of Miller but is not in contradistinction with his rather indistinct pictures nor with his description. This fact is of the greatest importance.

The constant endolymphatic hydrostatic pressure on the upward facing macula at the bottom will, namely according to our theory produce an upwards directed motor tendency of the gecko This upwards directed tonus reaction from the lagena, as well as from the other sensory epithelia of the labyrinth is in certain positions probably reinforced from the large endolymphatic sac (Hamilton) lying at the side of the neck with prolongations between the muscles here (Wiedersheim). The increased pressure from here comes into play when the head comes downward, a fre-

quent situation for these animals. This may also happen when the muscles of the neck become contracted. A help in hearing, as suggested, cannot be excluded.

The Sphenodon (fig. 56) has a lagena which according to Miller and to my photos from Groningen, seems to be of the same type as in most saurians. The Sphenodon or Hatteria, which is only mentioned in this place to keep the order of Retzius, is in reality a more primitive reptile than the saurians, being the only living species of an extinct independent reptile order the Rhynchocephalians. That it has left the water is seen from the rather considerable size of the macula utriculi (fig. 56) with distinct relations of dominance. The macula sacculi is very large but the sensory epithelium is reduced to two rather small upper and lower parts of equal height and extension, separated by a very broad *perfectly blind part (fig. 57). As the nerve is* running outwards and downwards, the lower part must be regarded as representing the domiannt. In the front the two separated parts seem to coalesce. The type is perhaps a further development of that described above in the Chelydra.

D Crocodiles.

Only one species, the Alligator mississipiensis, is represented by Retzius (fig. 60) as also between my own histological observations (fig. 55 B). The saccular as *well as the utricular macula are of moderate and fairly equal size. The saccular macula* is seen with an upper dominance corresponding to the upward direction of the nerve *with an indication of an intermediate neutral part. The lagena is situated at* the lower end of the cochlea. It forms a rather long tube directed downwards and *forwards, opening a little at the lower end. The inner is covered by its macula nearly* the whole way round except at the bottom and a part of its outer side. The macula lagenae *spreads somewhat more on the forward than on the backward part as found* on a histological preparate, horizontally cut, (L.), probably a general feature in this organ. *Dubious interdentations may represent the individual toes (?).*

V Birds.

The birds, near relatives of the reptiles, have also one nape-knot, and consequently a great mobility of their neck. Their very large semicircular canals have a strongly developed septum cruciatum on their vertical crests.

8 species are represented by Retzius. goose (Anser domest.)(fig. 61) goose-ander (Mergus Merganser), pewit (Vanellus crist), snipe (Scolopax rusticola) hen (Gallus domesticus) thrush (Turdus musicus), sea-eagle (Haliaetus albicans), all very uniform, as are the birds as a whole. A likewise very uniform series is described by Satoh (pigeon, parrot, starling, hen and duck).

The relation between the enormous flat horizontal macula utriculi already mentioned (fig. 62) and the very small, vertically standing saccular macula is very important for the general conception of the way of acting of the maculae, and further also for that of the crests and the organ of Corti. In the air most progressive

rectilinear movements are vertical, including the constant vertical influence of gravity Horizontal alterations are on the other hand negligeable. If as generally postulated the stimulation were produced by gliding and not pressure the maculae utriculi and the maculae sacculi ought to have interchanged as well as to size as to position, i.e. the general accepted conception is impossible. It is interesting to note that in an embryo penguine (L) a bird who cannot fly but only walk and swim, I found the saccular macula, in birds ordinarily measuring a few percents of the size of the macula utriculi, being here about 2/3 The semicircular canals were still well developed (diving?).

The lagena forms a long, downwards pointing tube. In a preparate by Satoh (fig. 64) the upper part exactly as in the lizard (fig. 59) is lined by the macula the whole way round except at the outer side. Further down this horse-shoe form of the sections opens more and more But at last again the bottom is perfectly covered (fig. 63 65 66) just as we have seen in the gecko Werner[5] (1940) is the only author putting stress of the unique upward facing of the bottom of the macula lagenae But he does not draw any physiological conclusion from this important fact. The ordinary form of this macula is reflected in its otolith (fig. 65). Has the narrowness of the bottom in the ostrich (fig. 66) anything to do with the disability in these animals to flight and with the strong development of their feet (running, swimming) ? It would perhaps be interesting to compare the development of the macula lagenae with the use of flight and of feet and toes in various birds. In the pictures of Satoh the characteristic dominance of the two other maculae is easily seen, though not noted in the text.

The pictures given by Retzius are not incompatible with this conception, but on the other hand not prooving of the real relations, and the text is silent. De Burlet[1] gives a false picture of the lagena of the pigeon presenting it with a nude bottom In the figure of Satoh it can be seen that the expanding of the macula is such that with an oblique direction and by not continuing the sections quite down to the bottom of the preparate it is possible to depict this as nude.

Benjamins and Huizinga found no influence on the flight of pigeons after ex tirpations of the lagena on one or both sides. The same experience was already made by Thornval.

In the birds the tegmentum vasculosom has undergone an enormous development. Identical formations, only on a smaller scale are also seen in the sacculus and in the utriculus underlining the necessity of an immediate regulation of the endolymphatic pressure in changing heights. The same formation is also seen in the lagena (Satoh).

VI Mammals

A. Monotremata

In the spiny ant-eater (Echidna aculeata) the mac.utriculi and mac.sacculi are of about the same size with very distinct difference of dominant, intermediate and subordinate parts (fig. 67).

The large lagena (fig. 68) forms a sack lying behind, to the inside and a little below the cochlea. The macula forms a rather broad circular ribbon, lining the middle of the interior facing more or less downwards nearly the whole way round. At the whole way round. At the lateral side the ribbon is, so to say cut and the two ends turned upwards into two hollow tops. The lagena of the Echidna is thus rather different from that of the birds. But in both cases, as also in the reptiles, the macula is more or less regularly or completely facing concentrically towards the downward pointing axis of the funnel or sac of the lagena, widening more or less downward (G).

The function of the Lagena.

We have already discussed this problem as far as the fishes and the amphibians are concerned. According to the anatomy and to our principles the macula lagenae, as constituting a part of a sphere, should react more or less to all rectilinear horizontal transverse as longitudinal accelerations. The hydrostatic endolymphatic continual pressure should release centripetal, rather horizontal motor reactions, combined by a more or less distinct downwards component, except in the gecko and in the birds. The tube-form of the lagena is most pronounced in the saurians, the crocodiles and particularly in the birds. It is, as already hinted at, wanting in the limb-less serpents and also according to Miller in the Diploglossus lessonae a relative of the limb-less slow-worm. This points to the sidewalls of the tube-formed lagena as representing the legs and probably particularly the toes.

Nearly all frogs and reptiles dig. They cling to the ground, the reptiles haking themselves firmly to their substrate by their strong, spread claws. The same is the case with the Echidna, who is an eminent digger with very strong digging feet and claws which enable it to disappear into the ground at a moments notice. Also its relative the duck-bill (Ornithorynchus), digs with feet especially adapted and also furnished with swimming web.

And now the birds, only very few dig. But they use their separated slender but strong toes and claws. (About 1 1/2 million years ago their ancestor the Ornithorynchus, had claws also on its forelimbs). Also modern birds need, however the clutching of their toes and claws for holding their prey for swimming, for scratching in the ground for food, or for balancing on a wavering branch, even during sleep *The movements needed for such manoeuvres of the toes are all concentric, centripetal.* In birds it should be noted that the alternating rhytmic moving of right foreleg and left hindleg and vice versa (strottings) has been broken, and therefore also the influence of the lagena upon flight. Notwithstanding the different appearence the anatomical-physiological principles are the same in reptiles, birds nad monotremata. The frog lagena is the first suggestion of the structure and function here described.

But why has it later disappeared? Perhaps because a certain type of extremities went out of fashion during the further evolution, a type with long slender toes, fur nished with strong claws, rather spread from each other bending and streching concentrically and relatively independently of the rest of the extremity One therefore

cannot but think over whether the macula lagenae already had disappeared in the extinct, huge, walking reptiles with their high legs and short and clumsy feet of support?

B Higher Mammals.

All mammals, also the monotremata, have two nape-knots. They therefore, have not the same mobility of their neck as the reptiles and the birds. They have also no septum cruciatum on their vertical crests. On the other hand probably in connection with the more horizontal way of moving, they have a planum semilunatum at both ends, also of the lateral crest.

The dominance of the maculae is only faintly visible in most mammals, as in the guinea-pig and the Lemmus (L). In the conny (Procavia) the macula utriculi, however shows a distinct anterior dominant middle neutral, and posterior subordinate part (fig. 70). Its macula sacculi shows in horizontal section (fig. 71) a distinct anterior somewhat curved, dominant part in contradistinction to a posterior subordinate part. The interesting is that we here as also in other cases (man swine (R)) and in the crests in the shark) find that the dominant part may be the shorter The increased curvature, however seems to give a higher selecting sensivity as to determining the direction of the impact.

This anterior part of the saccular macula »the saccular corner» of Magnus and de Kleyn furnished by its own nerve (the nerve of Oort) is often clearly separated from the rest as seen in swine and man (Retzius) In all mammals the saccular nerve branches are seen directed from below upwards, indicating the upper part as dominant. It should perhaps be noticed that already in 1951 Lorente de No has been on the way of discovering the rule of dominance, describing how in the mouse the nerve fibers in the macula utriculi were more strongly developed in the anterior and external zones, where also the otolith crystals were larger than in the external and posterior zones. In the middle the nerve fibres were smaller than elsewhere and the crystals were smaller and scanty A difference in size of the otoconia according to dominance is, by the way a widely spread histological finding which, quite incomprehensively has escaped general notice.

As the most typical terrestrial of all vertebrates, equally exposed to accelerations in all three spatial dimensions, the size of the two maculae is of fairly even size (»ground type») As in all other vertebrates the water species have comparatively large labyrinths, being without guiding orientation from touching the ground. The sea-lion has a larger labyrinth than has a camel (Gray) In the whales (fig. 71) the semicircular canals have become greatly reduced on account of the stiffness of their neck exposing them only very little to curved accelerations. The same but in lesser degree is seen in the Dugong who also is rather stiff-necked. The sea-lion has a very wide cochlear aqueduct (fig. 72) Perhaps it makes the surrounding water pressure if it becomes too large, act on the labyrinth via the intracranial and intraspinal fluid system and by increasing the intralabyrinthine pressure and thereby the tonus, forces the animal upwards again. The opposite condition is found in the monkeys and in man where the well-known narrowness of the cochlear aqueduct hinders the

frequently strongly changing body positions with varying intracranial pressure from influencing the labyrinthine function.

Already Retzius (fig. 73) did observe that the cat, an animal of excellent dexterity has a stripe devoid of sensory epithelium across its vertical crests. And in the bats where the semicircular canals are enormous, with crestal epithelia by far exceeding their maculae, Iwata found the vertical crests with rather high transverse ridges devoid of sensory epithelium and with cupulae tending to divide into two halves (fig. 74)

As far as may be seen from his illustrations the two maculae are fairly equal in size. This would mean that circular accelerations should be of greater importance for their flight than straight vertical ones as in the birds. I found, by the way in a young animal (L) the acoustic nerve of the double size of the vestibular in correspondence with their very sharp hearing, guiding them with echo signals. The vertical crestal ridges, which according to de Burlet[1] also may be encountered in marsupials and insectivores seem to speak here as in the sauropsides, against the existence of a special subcupular space. In the experiments of Jerlang in which by instillation of chloroform in the external meatus or injection of croton oil into the middle-ear an edemazation was produced in every potental fluid space of the labyrinth, a subcupular space also was never seen.

The question of the hearing capacity of the vestibular labyrinth has only been peripherically touched upon in the preceeding pages. That sound as consisting of rapidly and regularly changing oppositely directed rectilinear accelerations may be able to stimulate the vestibular neuroepithelium is in accordance as well with our theory as with a long series of facts, partly already mentioned. To these we may add the sensivity to sound of the saccular and probably also the lagenar macula in Acanthias (Vilstrup[1]), the sensivity of the front part of the frog saccular macula to mechanical vibrations (Ashcroft and Hallpike), and the remarkably excellent hearing of several fishes, also outside the ostariophysarians (von Disselhorst). And particularly just as in several mammals there is reason to believe that the deepest frequencies are not perceived by the organ of Corti (S.H. Mygind [11 12 13]), but in the bulla as mechanical vibrations, so a corresponding defiency of the hearing organ may be found in lower vertebrates. In the pigeon Rob L. Board and Grant L. Rasmussen have found the »cochlear» nerve dividing up in three isolated branches, starting from the basal, the middle and the apical parts of the spiral ganglion. This range of branches is regularly and in a perfectly identical way continued below into a fourth and fifth part for the lagena. This organ has besides a special relationship to the endolymphatic space in birds (de Burlet[1]). It is therefore likely that the lower frequencies in these and probably also in lower vertebrate species are not perceived as musical tones, but as mechanical vibrations of a more or less distinctly perceived special periodicity A puzzling fact has been drawn forward by Bocca and Sperani. Already several years ago Bocca has been able to demonstrate nervous connections between vestibular fibres and the cochlear nerve apparently explaining an existing limited acoustic reaction in patients otherwise apparently stone deaf but with normal vestibular reactions.

CONCLUSIONS

The fundamental factors for labyrinthine function are »pressure» and »dominance»

In this way the many anatomical variations of the vestibular labyrinth are found in harmony with the way of living and particularly of locomotion of each individual vertebrate species.

The dominant, more sensitive part of the sensory epithelium is always that which is supplied by the most peripheric part of the nerve the subordinate, less sensitive part being the opposite, often with an intermediate more or less »blind» or »neutral» part intervening. Similar dominance relations exist in the sense of touch of the skin, in the eyes and in the acoustic labyrinth. Dominance and »polarization» are different phenomena, but may in some cases coincide.

As in other senses of positional orientation the stimulation releases, besides a sensation, correcting motor reactions, directed in the axis of the individual cell stimulated and against the impact of the stimulus.

The combined motor reaction therefore depends on the degree of dominance the size and spatial position (»front») and the curvature of the individual epithelium.

The pressure causes a »downward» displacement of the sensory cell in relation to its stiffer supporting cell. By this »downward» displacement the giant hyaluronic acid molecules, supposed to be bridging between the two sorts of cells, become curbed. This curbing releases an electric output, stimulating the appertaining nerve.

An opposite displacement and therefore also an opposite curbing of the giant molecules is prevented by the oppositely wedge-shaped forms of the heads of respectively *sensory and supporting cells. The movements are of molecular dimensions* (modified theory of J.A Christiansen)

The tonus reaction is caused by the hydrostatic pressure of the endolymph on the maculae and crests, all facing more or less upwards and backwards, except the macula lagenae. The tonus counteracts the continuous influence of gravity and the tendency to topple by forward moving. The perception makes it theoretically possible for all vestibular epithelia to react not only to ordinary accelerations, but also to mechanical vibrations and to sounds, these consisting of rectilinear accelerations with opposite rapidly and regularly changing directions.

While in the numerous cases a difference of dominance according to rule was histologically verified, a difference in contradiction to the direction of the nerve was never found. In the crests it has not been possible histologically to demonstrate this directional difference opposite in the external and in the vertical canals, except in the lowest vertebrates, the Myxine and, less distinctly in the Acanthias.

In the utricular macula the dominance is the most evident, the anterior part being the dominant together with the outer part, the inner and back parts forming the subordinate and the middle the »blind» part. The utricular macula also presents a strong curvature except in birds. Only in the rays the anterior dominance changes over to becoming posterior corresponding to the special way of life of these animals.

In the macula sacculi the anterior part is always the dominant and the posterior the subordinate part, but the difference is here much smaller Also the curvature is less pronounced.

In the ostariophysarian fishes the macula utriculi and lagenae probably take over a part of the function of the macula sacculi, which has become a hearing organ.

Very characteristically the saccular dominance during evolution, starting with the saurians, changes over from inferior to superior contemporaneously with the changing demands of equilibrium. The extremities become at the same time longer and tooles of real walk.

The macula lagenae seems to be without dominance, a circumstance which seems to favour its possibility for perceiving the correct direction of vibrational stimulation (»pseudo-hearing»). An exceptional size of this macula was found in 3 fishes living in muddy water where the difficult orientation by sight may have lead to a compensating orientation by means of mechanical vibrations.

The outward facing of the macula lagenae in sharks and its upward facing in the bottom-dwelling rays point also to the existence of such »pseudo-hearing».

The extraordinary development of the maculae utriculi and lagenae in the only two electric fishes observed should be remarked.

In conformity with our theory it is easy to understand that in air-vertebrates (birds), exposed to vertical accelerations, the macula utriculi is large the macula sacculi small, while the opposite is the case in water vertebrates.

In the vertebrates clinging to the ground, using their feet and claws for digging, for catching their prey for sitting, the macula lagenae is particularly large, developing from the simple downwards and outwards directed cup like macula in frogs to the sac or tube more or less downwards widening in reptiles, lined by a centripetally and downwards facing the macula lagenae as also in the Echidna, a master digger

The lagena of the birds reminds very much of the reptile type with its downwards directed tube-like from and the centripetally facing macula. Only the macula here also covers the bottom, facing upwards. The same is also seen in the gecko indicating an upward directed motor tendency in both cases. The lagena disappears suddenly and perfectly traceless at the same time as a special type of feet with slender concentrically movings toes goes out of fashion.

The septa cruciata in the lamprey in the reptiles and the birds, the mentioned stripes in the cat and the ridged in the bats (all in the vertical canals) make the generally accepted idea of a gliding of the cupola impossible. The same may be concluded from the continuous common otolith membrane of the saccular and lagenar maculae in several plagiostomes and fishes.

The principles governing the vestibular function are thus the same as those stated in preceeding works on hearing and in reality the same as in other position regulating sensory systems (senses of sight, touch).

ZUSAMMENFASSUNG

Gestützt auf einem repräsentativen komparativ-anatomischen Material, darunter auch von eigenen histologischen Untersuchungen, wird gezeigt, dass ein genauer Zusammenhang zwischen jeden Einzelheiten des Labyrinthes und der spezielle Bewegungsform des betreffendes Wirbeltieres besteht. Dieses gilt nicht nur die Grösseverhältnisse des ganzen Labyrinthes, sondern auch die seiner einzelen Teilen. Entscheidende Faktoren sind weiter die Stellung im Raume der einzelnen Epithelien (»Front»), ihre Beugung und ihre »Dominanz». Diese äussert sich als einer Sensibilitäts-unterschied zwischen entgegensetzten Teile, wodurch der eine, der von den periphersten Nervenästen versorgt ist immer der meist empfindsame »dominant» ist, während der andere, weniger empfindsame »subordinat» ist. Dieser Unterschied ist an der Maculae sacculi und besonders an der Macula utriculi in der Dimensionen der Sinneszellen wie auch in der Nerveäste deutlich. Der dominante und der subordinate Teil ist oft von einem, was sowohl Sinneszellen wie Nerven betrifft, noch weniger entwickelten, intermediären oder blinden Teil geschieden. In den Cristen ist dieser Unterschied nur bei der Lamprete ausgesprochen und bei dem Acanthias angedeutet. Bei den übrigen Wirbeltieren zeigt er sich nur in der Richtung und Ausbreitung der versorgenden Nervenäste, die mit der entgegengesetzten Wirkungsgrösse von derselben Strömungsrichtung in der horizontalen und in den vertikalen Ampullen übereinstimmen. Das Septum cruciatum der Reptilien und der Vögel repräsentieren den blinden Teil. Dasselbe gilt den entsprechenden Querleisten der Fledermäuse und den Querstreifen der Katze, alle nur auf den vertikalen Cristen. Diese Eigentümlichkeiten scheinen eine Gleitung der Cupola unmöglich zu machen. Ein Subcupularraum gibt es kaum. Dominanz und Polarität sind nicht identisch, können aber mitunter zusammenfallen. Die Macula lagenae ist ohne Dominanz, ein Vorteil vermuhtlich für die genaue Richtungsauffassung mechanischer Vibrationen (»Pseudo-Gehör»). Von den Fröschen ab beginnt die Macula lagenae nach unten zu wenden Bei den folgenden Formen nimmt sie Gestalt eines Sackes, eines Trichters oder eines Rohres von der nach unten wendenden Macula oben geschlossen der geotrophischen Lebenswesen dieser Tieren entsprechend, und besonders ausgeprägt bei Schlangen und gliederlosen Saurien. Bei den Geckos und bei den Vögeln aber ist die Lagena von der nach oben gewendenten Macula unten geschlossen, ganz mit den geofugalen Lebenswesen dieser Tieren wieder übereinstimmend. Das Verschwinden der Lagena bei den Säugetieren oberhalb der Monotremata, ist wahrscheinlich durch den Wegfall eines besonderen Fusstypus verursacht, durch starke wohl geschiedene Zehen mit konzentrischen Bewegungen charakterisiert. Der Mechanismus der Auslösung der vestibulären Reaktionen ist eine Druckwirkung von den Membranen auf den Sinneszellen, wodurch diese zwischen den steiferen Stützzellen nach »unten» geschoben werden Deise Verschiebung verursacht eine Beugung der langen Riesenmolekülen von Hyaluronsäure welche der Verfasser sich als Brücken zwischen Haarzellen und Stützzellen ausgespannt denkt. Diese Beugung erlöst elektrische Potentiale die eine Stimulation der Nervenelemente hervorrufen. Die effektive Grösse diesen interzellurären Verschiebung ist von molekülären Dimensionen. Die Prinzippen der statischen Funktion stimmen mit den von anderen stellungsorientierenden Sinnen wie dem Sehen, dem Tastsinn und vor allem dem Gehör überein.

RÉSUMÉ

Une série assez representive des investigations d anatomie comparative partiellement personelles, revèlent une harmonie intime entre la structure detaillée du labyrinthe et la facon de se mouvoir de l espèce de vértebré individuelle Ce regarde les dimensions, non seulement du labyrinthe total, mais aussi de ses parts différentes. Facteurs décisifs sont la position dans l'espace de l'épithèle en question (son »front»), son courbement et sa dominance. Celle-ci se manifeste comme une différence de sensibilite entre des parts opposées, ainsi que l'une celle fournie par la part du nerf la plus périphérique, est toujours la plus sensitive »dominante» tandis que l'autre part est la moins sensitive »subordonnée. Cette différence est plus prononcée dans le saccule. Elle est visible anatomiquement par une différence des dimensions des cellules sensorielles et des rameaux nerveux appartenants Les parts dominantes et subordinées sont souvent separées par une part intermediaire ou aveugle avec des cellules sensorielles et des rameaux nerveux encore moins developpés. Dans les crêtes les differences correspondantes sont évidentes seulement chez la lamproie et encore visible peut-etre chez le requin. Dans les autres vertébrés la dominance dans les crêtes se manifeste seulement par la direction et la distribution de leurs nerfs, correspondantes aux effets différentes dans les ampoules verticales et horizontales. Le septum cruciatum des reptiles et des oiseaux, les crêtes transverses des chauves-souris, les lignes nues correspondentes chez le chat semblent de rendre un glissement de la coupole impossible. Elles representent toutes la part intermédiaire ou aveugle. Un espace sous-coupolair probablement, n existe pas. La macule de la lagena est sans dominance un avantage apparamment pour la perception de la direction précise des vibrations mécaniques. Dominance et polarisation ne sont pas identiques, mais les deux phénomènes coincident parfois. Dès les grenouilles la macule lagénaire commence a donner a dedans. Chez les ordres suivantes la lagena prend la forme d'une tube, d'un entonnoir ou d'un sac avec la macule donnante à bas (assez plate chez les serpents et certaines sauriens sans jambes) correspondante a la vie géotropique de ces animaux. Seulement chez les geckos et chez les oiseaux, la macule donne en haut, correspondante a leur vie géofugale L absence de la lagena chez les mammifères superieurs est probablement due a la disparition du type du pied speciale aux doigts forts et bien séparés, aux mouvements concentriques. Tous les épithèles vestibulaires réagissent aux vibrations mécaniques et possèdent, théoriquement, la faculté de réagir aux vibrations sonores. Le mécanisme de déclenchement des réactions vestibulaires consiste en une pression des membranes sur les cils des cellules sensorielles, les déplacante »en bas» en rélation de leurs cellules de support plus raides. Par ce déplacement les molecules gigantesques d acide hyaluronique, supposées de former des ponts entre les deux sortes des cellules épitheliales, deviennent courbees. Et ce courbément les fait émettre des potentiales électriques, qui déclenchent une stimulation nerveuse Le déplacement intercellulair est de dimensions moléculaires. La correspondance entre les principes gouvernants la fonction vestibulaire et ceux des autres sens d orientation de position, comme ceux de la vue, de l attouchement, et surtout de l'oui est frappante.

ZUSAMMENFASSUNG

Gestützt auf einem repräsentativen komparativ-anatomischen Material darunter auch von eigenen histologischen Untersuchungen, wird gezeigt, dass ein genauer Zusammenhang zwischen jeden Einzelheiten des Labyrinthes und der spezielle Bewegungsform des betreffendes Wirbeltieres besteht. Dieses gilt nicht nur die Grössevernhältnisse des ganzen Labyrinthes, sondern auch die seiner einzelen Teilen. Entscheidende Faktoren sind weiter die Stellung im Raume der einzelnen Epithelien (»Front«) ihre Beugung und ihre »Dominanz«. Diese äussert sich als einer Sensibilitäts-unterschied zwischen entgegensetzten Teile, wodurch der eine, der von den periphersten Nervenästen versorgt ist, immer der meist empfindsame »dominant« ist, während der andere, weniger empfindsame »subordinat« ist. Dieser Unterschied ist an der Maculae sacculi und besonders an der Macula utriculi in der Dimensionen der Sinneszellen wie auch in der Nervenäste deutlich. Der dominante und der subordinate Teil ist oft von einem, was sowohl Sinneszellen wie Nerven betrifft, noch weniger entwickelten, intermediären oder blinden Teil geschieden. In den Cristen ist dieser Unterschied nur bei der Lamprete ausgesprochen und bei dem Acanthias angedeutet. Bei den übrigen Wirbeltieren zeigt er sich nur in der Richtung und Ausbreitung der versorgenden Nervenäste, die mit der entgegengesetzten Wirkungsgrösse von derselben Strömungsrichtung in der horizontalen und in den vertikalen Ampullen übereinstimmen. Das Septum cruciatum der Reptilien und der Vögel repräsentieren den blinden Teil. Dasselbe gilt den entsprechenden Querleisten der Fledermäuse und den Querstreifen der Katze, alle nur auf den vertikalen Cristen. Diese Eigentümlichkeiten scheinen eine Gleitung der Cupula unmöglich zu machen. Ein Subcupularraum gibt es kaum. Dominanz und Polarität sind nicht identisch, können aber mitunter zusammenfallen. Die Macula lagenae ist ohne Dominanz, ein Vorteil vermuhtlich für die genaue Richtungsauffassung mechanischer Vibrationen (»Pseudo-Gehör«). Von den Fröschen ab beginnt die Macula lagenae nach unten zu wenden. Bei den folgenden Formen nimmt sie Gestalt eines Sackes, eines Trichters oder eines Rohres von der nach unten wendenden Macula oben geschlossen, der geotrophischen Lebenswesen *dieser Tieren entsprechend, und besonders ausgeprägt bei Schlangen und gliederlosen* Saurien Bei den Geckos und bei den Vögeln aber ist die Lagena von der nach oben gewendenten Macula unten geschlossen, ganz mit den geofugalen Lebenswesen dieser Tieren wieder übereinstimmend. Das Verschwinden der Lagena bei den Säugetieren oberhalb der Monotremata, ist wahrscheinlich durch den Wegfall eines besonderen Fusstypuss verursacht, durch starke wohl geschiedene Zehen mit konzentrischen Bewegungen charakterisiert. Der Mechanismus der Auslösung der vestibulären Reaktionen ist eine Druckwirkung von den Membranen auf den Sinneszellen, wodurch diese zwischen den steiferen Stützzellen nach »unten« geschoben werden Deise Verschiebung verursacht eine Beugung der langen Riesenmolekülen von Hyaluronsäure, welche der Verfasser sich als Brücken zwischen Haarzellen und Stützzellen ausgespannt denkt. Diese Beugung erlöst elektrische Potentiale, die eine Stimulation der Nervenelemente hervorrufen. Die effektive Grösse diesen interzellurären Verschiebung ist von molekülären Dimensionen. Die Prinzippen der statischen Funktion stimmen mit den von anderen stellungsorientierenden Sinnen wie dem Sehen, dem Tastsinn und vor allem dem Gehör überein

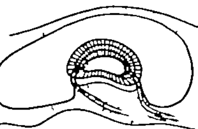
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Fig. 9



Fig. 10

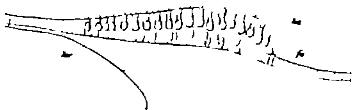


Fig. 11

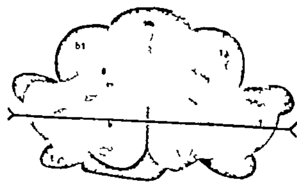



Fig. 16

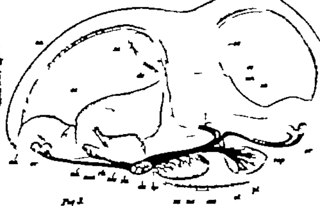
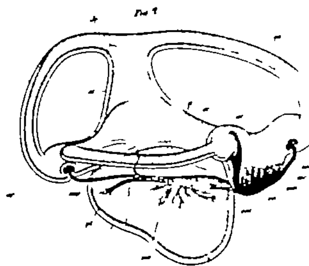
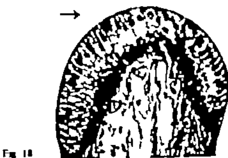
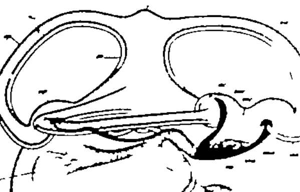
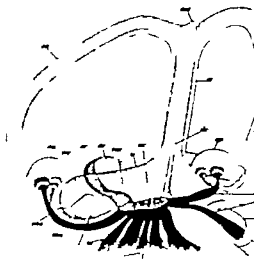

Fig. 20

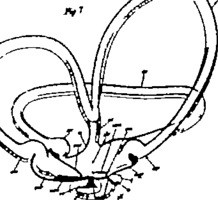

Fig. 17


Fig. 1


Fig. 18



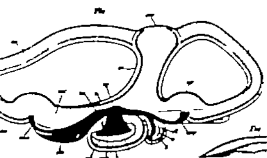




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Fig. 31



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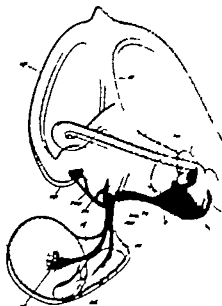
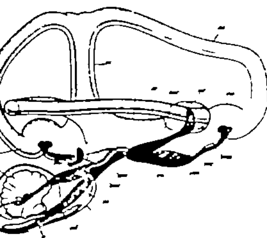
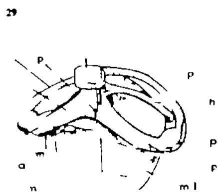
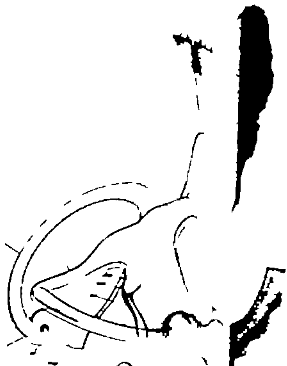


Fig. 32



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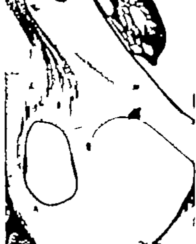


Fig. 58



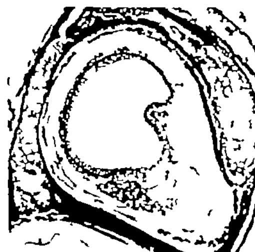
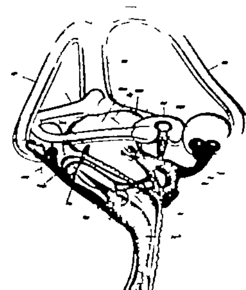
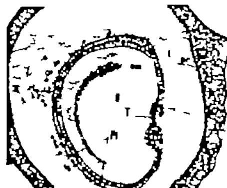


Fig. 59



Fig. 6





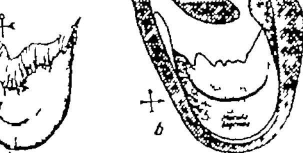


Fig. 65

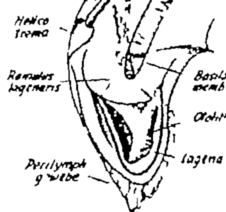


Fig. 66

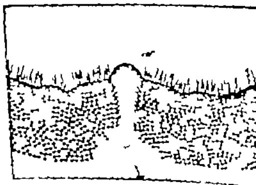


Fig. 69





Fig. 70







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An electromyographic and cineradiographic study

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SUPPLEMENTUM 250

From the Departments of Otorhinolaryngology and Clinical Neurophysiology of the University of Göteborg and from the Kresge Hearing Research Institute and the Department of Otorhinolaryngology of the University of Michigan Medical School

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ORSTADIUS BOKTRYCKERI AKTIEBOLAG
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CONTENTS

INTRODUCTION

A normal velopharyngeal closure mechanism is of the utmost importance in speech. We must be able to control the coupling gate between the nasal resonators and the oropharyngeal part of the vocal tract in order to speak distinctly. Inability to achieve closure may seriously distort speech and constitutes one of the major clinical problems in the field of phoniatrics and logopedics.

There is much information available on the articulatory movements of the soft palate and the velopharyngeal walls. They have been studied directly and indirectly by a number of means, such as direct observation from an antero-superior direction in patients with facial defects following tumor surgery, cinéradiography, aerodynamic procedures with air flow and air pressure measurements, and acoustic analysis of speech.

There is considerably less reliable information available to answer the question: How are these movements controlled by the various muscles involved? Here we are left mainly with assumptions and inferences from dissections, direct observations and motion picture analyses. We do not know definitely which muscles are active in the production of velopharyngeal closure. Nor do we know whether the opening of the velopharyngeal portal in speech is an active or a passive phenomenon.

The primary purpose of the present investigation was to produce evidence of the participation of individual velopharyngeal muscles in speech by means of electromyography. Special attention was paid to their different rôles in the production of oral and nasal sounds and to variations in their degree of activity for different vowels. These were the main objects of the first series of experiments.

The relationship between the electromyogram and the acoustic speech signal, however, is an indirect one. Therefore it was important to obtain information on the intermediate link, the movements of the articulators, which are produced by the muscle contractions and which shape the acoustic signal. The aim of the second series of experiments was to provide evidence of the movements of the soft palate by means of simultaneous cinéradiography. With this third parameter, time relationships between EMG, palatal movement and acoustic speech signal could be analyzed, and variations in the degree of EMG-activity compared with variations in palatal displacement.

I REVIEW OF THE LITERATURE

VELOPHARYNGEAL CLOSURE, AN HISTORICAL SURVEY OF CONCEPTS AND METHODS OF STUDY

In ancient Greece rhetoric was a greatly appreciated and fostered art Orators were held in high esteem and rules were laid down for effective public speaking This is also the age where we find the first signs of a scientific approach to the study of speech and voice Thus, Hippocrates (about 100 B C.) stated that when air is expelled from the body cavities, a sound is produced because of resonance within the head and the tongue articulates against the palate and the teeth to produce distinct sounds Aristotle (381—322 B C.) devoted a great deal of attention to speech and voice and their disorders. He located the origin of the voice in the trachea and larynx. He also recognized the difference between vowels and consonants. The distinction between nasal and oral sounds, however was not made in this period. Grammar still occupied a preeminent position, and attention was generally directed towards letters rather than speech sounds.

Galen (129—199) who had such a tremendous impact on medicine for more than a dozen centuries, also studied the larynx thoroughly by dissections and experiments on animals. He insisted that the voice was produced in the larynx, and he observed that pigs did not squeal after section of the recurrent laryngeal nerve In his writings there are references to a comprehensive treatise on the voice of which now only fragments exist, however Whereas Hippocrates and Aristotle refer to the uvula only as a seat for possible inflammation and oedema, capable of bringing about suffocation, Galen considered it of importance in speech, contributing to the volume and beauty of the voice and he compared it to a plectrum. He noted that persons who had their uvula cut off all the way to the base manifested a voice impediment

On the death of Galen in 199 A.D "the active prosecution of anatomical and physiological inquiry ceased absolutely The curtain descends at once and the Dark Ages have begun" as concluded by Singer (1925) From the "Dark" and Middle Ages there exists no evidence of progress in the study of speech and voice Thus Berengario da Carpi (1535) in one of the very first printed textbooks of anatomy dealt with the uvula in exactly the same way as the ancients.

With the Renaissance a rapid development took place In the manuscripts of Leonardo da Vinci (1452—1519) the soft palate *palato mobile* was mentioned and drawn for the first time as far as we now know Regarding its function Leonardo claimed that all vowels are formed with the most posterior part of the movable palate. His anatomical drawings and notes remained unpublished for nearly four centuries, however and he had no direct influence on the development of the anatomical and physiological sciences.

Falloppio (1561) professor of anatomy in Padua, presented the first publication in which the soft palate was described and distinguished from the hard palate Moreover he stated that luetic disease of the uvula alone does not affect the voice the soft palate must also be engaged a perforation through the palate is very harmful to the

voice. Fabricius ab Aquapendente, the famous follower of Falloppio in Padua, published a treatise on speech and its instruments (1603) where he discussed the production of speech sounds at length (and quoted Aristotle extensively). He observed that when sound passes through the nose the vowels lose much of their quality. He did not distinguish between nasal and oral speech sounds, however, and movements of the soft palate were not discussed.

It appears that the function of the soft palate and velopharyngeal closure were first recognized in the seventeenth century, possibly by pioneers in the education of the deaf. Thus, Amman (1700) stated that the soft palate regulates the passage of voice through the nostrils, "for when we wish to emit voice or breath through the mouth only, which may happen in the pronunciation of the letters which in the following chapter I shall name explosives, we shut the passage of the nostrils as with a valve, ..." He also evidenced a clear understanding of those pathologic phenomena of speech which we now call hyper- and hyporhinolalia.

Von Kempelen (1791) in his famous book on the mechanisms of speech and on his speaking machine gave an accurate account of the rôle of the soft palate in speech. He established that the speech sounds or letters *m* and *n* are the only ones to be regularly pronounced through the nose. He stated that closure is accomplished by the apposition of the soft palate to the posterior pharyngeal wall. He also observed that when a vowel is followed by an *n*, it is pronounced with an open passage to the nasal cavities.

Dzondi (1831) wrote the first monograph on the soft palate. In this he objected to the claim made by several authors of this period that the soft palate was elevated to such an extent as to occlude the choanae. He tried to get additional information on the movements of the soft palate by palpation. His studies led him to believe that the soft palate remained motionless during the formation of vowels, even though he was well aware of the fact that almost all older and contemporary physiologists were of the opposite opinion.

Apparently Hilton (1836) was first to report a study of the palate from above in a subject with a facial defect. His description of velopharyngeal movements during breathing, swallowing and speech includes observations such as the following: "Upon taking a full inspiration through the mouth the palate is directed more completely upwards and backwards and adapts itself to the advancing pharynx. This adaptation remains until the expiration has nearly terminated, but it should be remarked, that the sides of the pharynx do not, even in this case approximate so much as during deglutition." He noted that the soft palate is elevated during speech "to direct the air in the expiration through the mouth, and to prevent its passing through the nose ..." Two years later Bidder (1838) published a report of the same kind, obviously unaware of Hilton's study. He reaffirmed that the soft palate is elevated during speech, but arrived at the conclusion that this is an accompanying movement only, either completely insignificant or of secondary importance to that of the tongue. He believed that velopharyngeal closure occurred exclusively during swallowing.

Patients with maxillo-facial defects after tumor surgery still constitute one of our most important sources of information on the movements of the soft palate and pharyngeal

walls Following Hilton and Bidder a great number of studies on such patients have been published, lately in the form of detailed analyses of motion picture films, as will be related in a following section

The middle of the nineteenth century represents the beginning of a new era. Experiments were undertaken to give indirect evidence of velopharyngeal closure during speech. Brucke (1856) studied the emission of air through the nostrils with the flame of a candle. Czermak (1857 1858, 1869) devised several means to demonstrate movements and closure in the velopharynx Following a procedure used by Del rou (1841) in a study on swallowing, he introduced a specially modeled iron wire along the floor of the nasal cavity on one side and observed the deflections of its outer end brought about by the elevation of the soft palate By means of this simple mechanical device he was able to demonstrate the differences in palatal movements and positions during the production of different vowels the deflection of the iron wire being greatest for /i/ and smallest for /a/ A second procedure was the instillation of water in the nasal cavities during the production of sustained sounds. He noted that for /a/ the water tended to pass down into the lower pharynx more or less quickly while for other vowels tested it was kept in the epipharynx. By a third procedure, a cold mirror of glass or metal held under the nostrils, he demonstrated the absolute closure of velopharynx during the production of pure vowels as distinguished from nasalized vowels. Finally for demonstration before large audiences he used a pressure-sensitive system in the form of a rubber tube from one nostril leading to a tambour On top of the tambour was a small mirror deflecting a beam of light towards the ceiling. Pressure changes within the nasal cavities were thus made clearly visible to the whole audience in the form of movements of the spot light in the ceiling In this way Czermak (1869) was able to confirm and demonstrate his earlier findings of an increased elevation of the soft palate in the series of vowels /a/ /e/ /o/ /u/ /i/

Passavant (1863) repeated and confirmed some of Czermak s tests and also reported on another series of experiments, carried out in collaboration with Moritz Schmidt, who had a very insensitive pharynx Thus, they were the first to report on evaluation of velopharyngeal closure by means of posterior rhinoscopy Finding that during this examination there was not always a complete closure during the production of /a/ /o/ and /u/ as their next experiment they passed a thread through the nasal cavity around the velum and out again through the mouth By pulling the two ends of the thread they caused an insufficiency of the closure and they found that all speech sounds could still be produced correctly if the opening was not too large. As their final step they interposed 5-cm long rubber tubes of different diameters between the soft palate and the posterior pharyngeal wall. The use of a 6.8 mm thick tube with an inner cross-sectional area of 12.6 mm² did not appreciably influence speech. A 10 mm thick tube with an inner cross-sectional area of 28.3 mm² gave most of the consonants a nasal character but the vowels were still not influenced

In thi same report Passavant (1863) presented his observation of a horizontal forward bulging of the posterior pharyngeal wall during speech in a cleft palate subject He also found that simultaneously the lateral pharyngeal walls were adducted and th medial borders of the cleft velum approached each other He assumed that the horizontal ridge is formed also in normal subjects during speech, though con

cealed by the soft palate and thus not seen. This theory of Passavant soon met with opposition, as described by Smith (1964).

Gentzen (1876) made the first recordings of the palatal movements by means of a kymograph, a mechanical transmission system and a lever on the upper surface of the soft palate in a subject with a maxillo-facial defect. He confirmed Czermak's findings of different degrees of palatal elevation for different vowels, and so did Voltolini (1879) by means of a delicate instrument for anterior rhinoscopy.

Hartmann (1880) studied the firmness of velopharyngeal closure by imposing a pressure in the nasal cavities during speech with nasal olives and a rubber balloon. He measured the pressure needed to overcome the closure mechanism and found variations between different sounds and between different speakers. Vogel (1881) in a subject with a considerable defect of the nose placed a thin rubber balloon filled with water in the epipharynx and recorded the pressure changes in this system brought about by the movements of the soft palate.

Gutzmann (1891) described a procedure for clinical evaluation of velopharyngeal closure by auditory cues still widely used. The patient is asked to produce a series of alternating /a/ and /i/-sounds. If a change in vowel quality is noted when his nostrils are pinched this indicates an insufficient velopharyngeal closure.

With the discovery of x-rays at the end of the nineteenth century a new era was started. Radiography offered new possibilities to study the vocal tract. Scheier (1898) published the first report on the use of x-rays in the study of speech and voice. He described the appearance of the soft palate during fluoroscopy and was able to confirm earlier findings of velopharyngeal closure and of a varied elevation of the velum in speech. In the following decades improved x-ray techniques were accompanied by an increasing number of studies using single-exposure radiograms in speech research and speech pathology. Gottheimer (1929) presented the first cinéradiographic film of the vocal tract during speech. Carrell (1952) described a method for reduction of the radiation effect and advocated the use of cinéfluorography for clinical assessment of velopharyngeal closure. Cooper & Hoffmann (1955) and Skoog & Nylén (1955) were the first to report studies of the velopharynx by means of cinéradiography with image intensifiers. Bringing the radiation effects down to insignificant levels, this technique proved a most valuable tool, and it rapidly gained widespread use as described and exemplified by Croatt & Croatto-Martinolli (1959) and Truby (1959). In a study on vowels and disyllables, Moll (1960) presented measurements of some oral and velopharyngeal structures from cinéfluorographic frames as a function of time and he also related his findings to the frequency variation of the second formant of the vowels. Björk (1961) studied connected speech in normals by means of cinéradiography and synchronized speech spectrography and Nylén (1961) used the same procedures in a series of cleft palate subjects.

Thus in modern research on the soft palate and velopharyngeal closure, cinéradiography in lateral projection plays a very important rôle. Valuable indirect information is also provided by refined procedures and electronic recording equipment for measurement of nasal flow and recordings of oral and nasal air pressure during speech as shown by Warren & DuBois (1964) and surveyed by Hardy (1965).

Moreover an acoustic analysis of speech can be used to supply information relevant to nasal coupling, as described by Fant (1960)

In our present-day concept of the velopharyngeal closure mechanism during speech, the following components may be distinguished

1) elevation and posterior movement of the soft palate
2) forward bulging of the posterior pharyngeal wall,
3) medial movement of the lateral pharyngeal walls.

The elevation and posterior movement of the soft palate obviously gives the most important contribution to closure. It can be very clearly demonstrated by fluoroscopy in a lateral projection, and it accounts for the fact that many authors refer to velopharyngeal closure as a valve function

The forward bulging of the posterior pharyngeal wall was formerly considered very pronounced and essential to speech, notably in the form of "Passavant's ridge" This ridge was held as evidence of a circular constriction effecting velopharyngeal closure, and the concept of the "palatopharyngeal sphincter" was introduced. Calnan (1957) has thoroughly and most convincingly disputed the validity of Passavant's ridge in speech, however and in a laminagraphic study by Hagerty *et al* (1958) on 80 normal subjects this ridge occurred in 9 subjects only and in 6 of these "velar contact with the posterior pharyngeal wall was made above the ridge and so strongly that it appeared Passavant's bar was not necessary for functional closure" Hagerty & Hill (1960) measured the forward displacement of the posterior wall in 50 normals and in 50 cleft palate subjects phonating /a/ and /s/ and concluded "For both clefts and normals the actual amount of forward excursion of the posterior pharyngeal wall is very small and its contribution to speech is probably insignificant."

The medial movement of the lateral pharyngeal walls in speech is well known to those engaged in the rehabilitation of cleft palate speakers, since it is very marked and easily observed through the mouth during the phonation of /a/ in some of these subjects This movement no doubt represents an important compensation for a dysfunction of the soft palate Its occurrence in normal speakers is more difficult to demonstrate As mentioned, already Hilton (1836) described this approximation of the lateral walls of the upper pharynx in his subject with a maxillo-facial defect, and so did a great number of followers studying subjects of this kind. In Bloomer's tracings from cinéfilms (1953) it is clearly illustrated Harrington (1944) studying a cleft palate subject, claimed that the "mesial" movement of the lateral pharyngeal walls occurs over a great vertical distance and moreover that "the extent of mesial movement bears a direct relationship to the extent of velar elevation during velopharyngeal closure" He rejected the idea that the velopharyngeal mechanism is in the nature of a simple sphincter" Astley (1958) studied the movements of the lateral walls of the upper pharynx in 1 normal subjects by means of cinéradiography He used a postero-anterior projection and produced an outline of the lateral walls by installation of radio-opaque material through the nose He found that during the phonation of "ah" the lateral wall showed a pronounced inward shift, with maximum at about the level of the palate, where they touched together Speech was accompanied by to-and-fro movements at the same level, Campos-Ciral & Cole (1962) attached small balloons coated internally with radio-opaque material, to a palatal retainer and with those balloons

placed in the nasopharynx they made frontal-plane radiographs in 4 cleft palate subjects. In this way they assessed the extent and nature of pharyngeal wall movement during phonation. They remarked upon the finding of asymmetries and lateral rather than mesial movement in some patients. Björk (1961) obtained information on the coupling gate between the oral and nasal cavities in 10 normal subjects by means of tomography in an antero-posterior projection. He found that during intonation of various nasalized sounds the size of the cross-section varied and that the area diminished with increasing velopharyngeal closure in both frontal and sagittal planes. However the area was found to be in a linear relationship to the sagittal minor axis — the distance between the soft palate and the posterior pharyngeal wall. If this is true, the changes in the frontal axis length — from side to side — must be insignificant.

Thus it appears from a survey of the literature that velopharyngeal closure in speech is mainly brought about by the elevation and posterior movement of the soft palate. The forward bulging of the posterior pharyngeal wall is normally very small. Our knowledge about the extent of inward movement of the lateral pharyngeal walls is very limited, and the available evidence is somewhat contradictory.

MUSCLES OF THE SOFT PALATE

The palatal and pharyngeal muscles were first described by Italian anatomists in the sixteenth century. In the plates, completed in 1552, which Eustachio intended for a textbook of anatomy the tensor and the levator of the soft palate, the palatopharyngeus, the salpingopharyngeus and the constrictors can all be recognized. This anatomical and graphic masterpiece of Eustachio was not published until 1714 however and the text is lost. The first printed description of these muscles was presented by Falloppio (1561) who distinguished three pairs of muscle of the fauces, viz., those which we now know as the tensors of the palate, the constrictors, and the levators, to which he also attributed the muscle fibers of the palatal arches. In the seventeenth century anatomists devoted a great deal of attention to the muscles of the palate and pharynx. Thus Valsalva (1704) gave a description that can almost compete with modern textbooks. He was the first to claim that the tensor of the soft palate functions as a dilator of the Eustachian tube. His follower Morgagni (1740) commented upon Valsalva's treatise in a series of "epistolae" where the contemporary literature on the subject was thoroughly surveyed, the confused terminology analyzed, and the descriptive anatomy of the palatal muscles discussed at length.

In the nineteenth century Dzondi (1831) was the first of a series of German authors to engage in the study of the palatal muscles. As mentioned earlier in the first monograph devoted to the soft palate he reported on dissections and on examinations by palpation. He described in detail the anatomy and function of each of the muscles of this region. The importance of the superior constrictor in speech was pointed out by Passavant (1863, 1869). Luschka (1868) on the other hand, who published an extensive treatise on the human pharynx with many illustrations, upheld the importance of the palatopharyngeus muscle in velopharyngeal closure and considered the constrictor of very little significance. In the middle of the nineteenth century a great

deal of attention was also directed towards the function of the Eustachian tube, and among others Von Tröltsch (1861) made a careful study of the structure and function of the tensor and the levator as related to the opening and closure of the tube.

It is difficult to visualize the anatomy of the palatal muscles by reading and studying illustrations alone. Thus, in our century most writers in the field seem to have felt a need for making dissections of their own in order to arrive at a satisfactory three-dimensional concept. Dissections of palatal muscles have been reported by a great number of authors. Only a few of these have given a full account of their findings however and the reports are not in total agreement. To a certain extent this disagreement might be due to anatomical variations such as have been described by McMyn (1940), Harrington (1944) and Bosma (1953), variations that on some points seem to be considerable. But there is also a difference of opinion as to classification and naming of certain muscle structures.

So far as the movements of the soft palate and velopharyngeal closure in speech are concerned, the present concept of the action of the individual palatal muscles is mainly based on information from dissections of cadavers, correlated with direct observation of palatal movements in living subjects.

Among modern authors McMyn (1940) has given a thorough account of 6 dissections in this area, with emphasis on the salpingopharyngeus muscle. Harrington (1944) has related findings from dissections of 10 cadavers to measurements on palatopharyngeal configurations during phonation in 5 subjects. These measurements were obtained from motion picture films of one subject with a facial defect after maxillo-facial surgery and of one subject with a non-operated cleft of the palate, as well as from radiographs of 3 cleft palate subjects. Bosma (1953a) has dissected 18 cadaver specimens and correlated his anatomical observations with cinematographic studies of phonation and swallowing in 7 subjects — 6 with facial defects after tumor surgery and one with a wide cleft of the palate. Bloomer (1953) in his very detailed analysis of motion picture films from 2 subjects with facial defects, has also been able to draw certain inferences regarding the palatal muscles engaged in speech, swallowing and other activities.

A study of the literature has led to the following description of the palatal muscles and of their assumed functions, especially during speech (Fig. 1).

MUSCULUS TENSOR VELI PALATINI

Anatomy. The tensor is a flat muscle of triangular shape with its base along the anterior wall of the cartilaginous Eustachian tube, and its apex at the pterygoid hamulus. Arising partly from the spine of the sphenoid bone and the scaphoid fossa of the pterygoid process, and partly from the anterior wall of the tube, it descends almost vertically postero-medial to the medial pterygoid muscle and antero-lateral to the levator veli palatini muscle. Its tendon makes a right angle turn around the hamulus, and spreads horizontally or slightly upwards forming the aponeurosis of the soft palate to which the other palatal muscles are attached.

The tensor is of a complex nature and consists of differently structured layers, where muscle and tendon fibers have a varied distribution. Some authors, like Terracol *et al* (1949) recognized a superficial and a deep layer. Korner (1941) identified an outer

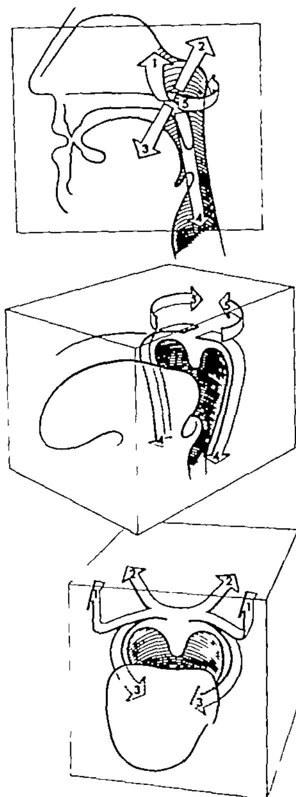


Fig. 1. Schematic presentation of the velopharyngeal muscles. The arrows indicate the approximate direction of their action and influence on the soft palate. 1. Tensor. 2. Levator. 3. Palatoglossus. 4. Palatopharyngeus. 5. Superior constrictor.

a middle and an inner layer while Deuschle *et al* (1960) in a study on cleft palate fetuses divided the muscle into a posterior an intermediate and an anterior part. The origin of muscle fibers from the hook of the auditory tube cartilage is generally agreed upon, while the origin of muscle fibers from the membranous wall of the tube has been disputed Thus, McMyn (1910) found that the tensor "usually arises from the posterior half of the membranous wall of the tube" but Körner (1911) denied this relationship Rich (1920a) and Korner (1911) supported earlier authors by reporting the insertion of fibers on the hamulus, while McMyn (1910) found little signs hereof

The thickness of the fibers of the tensor muscle was studied by Körner (1911) who found that they were of approximately the same diameter as those of the trapezius muscle of the back. In his microscopic sections from the tensor he also observed muscle spindles, as did Winckler (1961)

Innervation The mandibular branch of the trigeminal nerve which innervates the muscles of mastication also carries efferent motor fibers for the tensor veli palatini This was clearly demonstrated in dogs by Rich (1920b) who stimulated the cranial nerves and observed the exposed tensor The nerve supplying the medial pterygoid muscle gives off a small twig entering the lateral aspect of the tensor posteriorly according to Broomhead (1951)

Function. Some textbooks still classify the tensor as an elevator of the soft palate, but most modern researchers agree that, when the tensor muscles contract, they depress the anterior part of the velum. They also make it tense and effect an opening of the Eustachian tubes Contraction of the tensors is known to occur during swallowing In dogs, Rich (1920a) elicited the swallowing reflex and observed contraction of the tensor with accompanying opening of the Eustachian tube When stimulating the tensor electrically he also noted "a tension of the fibrous expansion of its tendon in the soft palate It can be seen that this latter effect does not actually modify the condition of the soft palate very much, for it appears to be concerned chiefly with that portion which is immediately adjacent to the hard palate — the so-called fibrous prolongation of the hard palate and this fibrous prolongation possesses a definite stiffness even when the tensor palati is at rest." When viewing the palate from above in a human subject with a facial defect Wardill & Whillis (1936) noted that during swallowing "dimples appeared on each side indicating that the tensors were contracting to flatten and lower the level of the palatal aponeurosis, and in this way to push the bolus on its journey In no other movement of the palate was any action of the tensors seen."

The contribution of the tensors to speech is doubtful. Rich (1920a) found no evidence of contraction of the tensors during "simple elevation" of the velum. Wardill & Whillis (1936) concluded that "the tensor palati would appear to have little to do with the speech mechanism, its activity being strongest at the time of deglutition, " Bloomer (1953) however registered "tensor depressions" in the anterior part of the soft palate in one of his two subjects "during speech and blowing as well as during deglutition, suggesting that the action of the tensor is synchronized and coordinated with that of the levator and other pharyngeal muscle groups" Calnan (1953) also studying a subject with a facial defect, noted that during speech "the action of the

tensor palati muscles could be seen tensing the anterior half of the palate then followed a little preliminary elevation of the palate, and finally the normal full elevation as the word was uttered." Studying the movements of the soft palate in speech by means of radioscopy Wilms (1953) inferred a synergistic activity of the tensor and palatoglossal muscles during the formation of nasal sounds.

MUSCULUS LEVATOR VELI PALATINI

Anatomy. The levator is a slender muscle of even dimensions along its course from the base of the skull to the palate. It arises from a small area in front of the canalis caroticus, and descends in a fronto-medial direction along the posterior and inferior border of the Eustachian tube. It inserts into the middle third of the soft palate where its fibers spread, meet the levator fibers from the opposite side and interlace with the other palatal muscles, particularly the palatopharyngeus. The levator is one of the least variable muscles in the pharynx, according to Bosma (1953a).

When measuring the cross-sectional dimensions of the levator fibers in microscopic sections, Körner (1911) arrived at figures close to those for the facial and ocular muscles, well below the corresponding figures for the tensor. In spite of considerable effort he was unable to identify muscle spindles in the levator, nor could Vidic do so, according to Winckler (1961).

Innervation. The levator is innervated by the pharyngeal plexus, commonly held to supply all the palatal and upper pharyngeal muscles except the tensor and the uvular muscle, according to Broombead (1951). The pharyngeal plexus is composed of branches from the glossopharyngeus, the vagus and the sympathetic trunk.

There has been a great deal of disagreement about the innervation of the levator as reviewed by Gowerez (1919) and Vidic (1964). It now seems well established, however, that the motor fibers to the levator leave the skull with the bulbar portion of the accessory nerve, as demonstrated in dogs by Rich (1920). These fibers join the vagus very soon after leaving the skull. In dissections on human cadavers Broombead (1951) found that a twig from the pharyngeal branch of the vagus enters the levator on the lower posterior border after passing over the superior constrictor muscle. According to Vidic (1964) fibers from the glossopharyngeus consistently join the vagus fibers assigned to the levator.

Function. By contraction of the levator muscles the mid-portion of the soft palate is moved upwards and backwards. The levator has a crucial influence on the position of the soft palate. It is therefore no doubt the most important of the muscles accomplishing velopharyngeal closure in speech, as shown by Bosma (1953b, 195) in his studies on patients with sequelae from bulbar poliomyelitis.

MUSCULUS PALATOGLOSSUS

Anatomy. The palatoglossus is a small muscle 1.5 mm thick and 3 mm broad at the level of the anterior pillar according to Luschka (1868). Arising from transverse fibers within the tongue it ascends in the anterior pillar of the fauces to the palate. Its fibers insert on the lower side of the palatal aponeurosis. There are no major discrepancies in the descriptions of the palatoglossus, and it has received little attention by modern researchers.

Innervation. The palatoglossus is supplied with motor fibers from the pharyngeal plexus according to Gowerez (1919) and Broomhead (1951).

Function. When the palatoglossus muscles contract, the palate is lowered and drawn forwards. It acts as an antagonist to the levator as pointed out by van Gelder (1965). It also participates in the elevation of the posterior part of the tongue. According to Negus (1918) it effects closure of the retro-oral portal, preparatory to swallowing. Bosma (1957), however, attributed this activity mainly to the tensor veli palatini. As mentioned above, Wilms (1953) inferred that the palatoglossus muscles, in cooperation with the tensors, are active in the production of nasal sounds.

MUSCULUS PALATOPHARYNGEUS

Anatomy. The palatopharyngeus is considerably larger than the palatoglossus. It has a wide origin from the back and side walls of the pharynx and from the thyroid cartilage. Its fibers converge towards the palate. The vertically oriented, anterior fibers of the muscle constitute the stroma of the posterior pillars. Inserting into the palate posteriorly and laterally, its fibers are divided into two strands by the descending levator muscle.

There are many variations and considerable disagreement in descriptions of the palatopharyngeus muscle. Some authors, like Wood Jones (1910), distinguish two separate muscles, the palatothyreoideus and the "true" palatopharyngeus, the former anterior passing from the larynx in the posterior pillars to the palate, the latter embracing the pharynx and giving rise to a third fold behind the pillars of the fauces. A variable number of fibers from the palatopharyngeus also insert into the tip of the medial cartilaginous wall of the Eustachian tube. They may be regarded as forming a separate muscle, the salpingopharyngeus, described in detail by McMyn (1910). "This muscle produces a sharp ridge concave medially on the lateral pharyngeal wall of the pharynx, just below and behind the tube" according to Wardill & Whillis (1936), the salpingopharyngeal fold. The caudal extension of this fold — visible in normal subject during gagging and in some cleft palate subject when phonating "ah" — is probably identical with "the third fold" of Wood Jones (1910) mentioned above, "which marks the true palatopharyngeus."

There are divergent opinions regarding the upper margin of the palatopharyngeus insert on on the back wall of the pharynx. Thus following Luschka (1868) most text books of anatomy indicate that it is at the level of the hyoid bone, whereas Wood Jones (1910) insisted that the upper fibers of the palatopharyngeus have a nearly horizontal course. As Bosma & Fletcher (1962) pointed out, there is an intimate and intricate relationship between the palatopharyngeus and the superior constrictor at this level. Townshend's description (1910) is graphic: "The muscles on the posterior pharyngeal wall are not as distinct and separate as the diagrams in the text books of anatomy would have one to believe. The fibers of the superior constrictor and the palatopharyngeus are often so hopelessly entangled in the human subject that it is impossible to say where one muscle begins and the other end. It can definitely be stated, however, that the fibers that form the highest part of the muscular coat of the pharynx generally arise from the palate and if they do not, the fibers situated immediately below them have this origin. These fibers take a definitely upward course

when passing from the palate to the pharynx inferiorly they are on the same plane as and intermingled with, fibers that pass horizontally round the pharynx from the palat and these again are blended with fibers that pass more and more obliquely downwards till the most anterior ones form the post rior pillars of the fauces."

Innervation. The palatopharyngeus derives it motor nerve supply from the pharyngeal plexus, according to Gomerez (1919) and Broomhead (1951)

Function. When the palatopharyngeus muscles contract the soft palate is pulled in a dorso-caudal d rection, the post rior pillars are stretched and adducted, the lateral walls of the upper pharynx are brought medially and the larynx and pharyngeal walls are elevated.

The palatopharyngeus obviously plays an important rôle in swallowing as demonstrated a series of reports by Bosma (195 a.o.) It can also be considered commonly accepted that the palatopharyngeus is acti in the production of velopharyngeal closure dur ng speech. The simultaneous contractions of the palatopharyngeus and the levator muscles position the soft palat horizontally an nterpretation brought forward by Luschka (1868) reemphasized by Podvinec (1952) repeated by Bloomer (1953) and stressed by Bosma & Fletcher (1962) The latter authors also rgued that, i phonation, "the apposition of the pharyngeal wall and dorsal aspect of the palate is commonly on rapid temporal schedule By anatomic inference it is an action of the nner and most medial portion of the palatopharyngeus.

MUSCULUS CONSTRICTOR PHARYNGIS SUPERIOR

Anatomy The superi r constrictor constitutes the muscular coat f the upper pharynx, f rming a broad band loop open a teriorly Its fiber are oriented in a mainly horizontal d rection. Four parts re distinguished. Accordinge to classical descriptions *Pars pterygopharyngea* is attached t the low half of the medial pterygoid plat and to the hamulus. *Pars buccopharyngea* inserts nto the pterygo-mandibular raphe *Pars mylopharyngea* attached to the inside f the mandible posteriorly *Pars glossopharyngea* inserts into the tongue The most cranial f these, Pars pterygopharyngea, is the one most closely related to elopharyngeal closure

Whillis (1930) described a palatal origin of superior constrictor fibers and suggested name for this portion, the palato-pharyngeal sphincter" In none f his specimens could any constrictor fibers be found attached to the medial pterygoid plat the bony attachment f these fibers at this level was lim ted to the hamulus. Harrington (1944) found constrictor origi in the palate in 8 dissections out of 10. H also reported find g a consistent interconnection between the palatopharyngeus and the pterygopharyngeus muscles. Thi occurred by means f many ind idual fibers rather than through large separat strand. I most cases the connection between the two muscles was so close that it was almost imposs ble to separat the two." Bosma (1953a) emphasized the ural ity f the constrictor musculature particularly f its anterior origin, but also of it degree of accentuation in the lateral and posterior walls at the level of the palat

Innervation The superior constrictor deri es its motor nerv supply from the pharyngeal plexus accordi g to Gomerez (1919) and Broomhead (1951)

Function. When the superior constrictor contracts, the upper pharynx is narrowed. This contraction is no doubt most effective in the lateral pharyngeal walls but it also gives rise to a slight forward bulging of the posterior pharyngeal wall. In some subjects this takes the form of a transversal fold, "Passavant's ridge." The palatal insertions exert a backward pull on the soft palate. In swallowing, the superior constrictor plays a very important rôle as shown by Bosma (1953b). In speech its importance has been advocated by many authors since Passavant (1863), among them Wardill & Whillis (1936), Harrington (1944), Bloomer (1953) and Calnan (1953).

When scrutinizing the literature on the functions of the superior constrictor and palatopharyngeus muscles, one must conclude that the available material is confusing. It seems wise to take the standpoint of Bosma & Fletcher (1962): "Thus, a difference of opinion exists as to whether the convergence of the pharynx about the palate represents action of the palatopharyngeus or of the constrictor. When we note that in the human and in a variety of subhuman species the palatopharyngeus originates at the dorsal and lateral walls of the pharynx and migrates toward the palate in close juxtaposition to the constrictor from which it originates, it can be seen that there is little point of profit to attempted embryologic, mechanical or nosologic distinctions of this sort."

MUSCULUS UVULAE

Anatomy. The uvular muscle is a paired, slender muscle, constituting the mid-line structure of the soft palate under its nasal mucosa. It arises from the back of the hard palate and overlies the other palatal muscles on its way to its insertion into the tip of the uvula. According to Voth (1961) some fibers from the palatopharyngeus muscle pass over the uvular muscle posteriorly.

Innervation. The uvular muscle derives its nerve supply from the lesser palatine nerves of the facial nerve, according to Brombead (1951).

Function. When the uvular muscles contract they shorten the uvula. The significance of the uvular muscle function is doubtful. There is no information on its possible participation in speech.

ELECTROMYOGRAPHIC STUDIES OF THE PALATAL MUSCLES

In the study of speech and voice production EMG has attracted considerable interest in recent years. From acoustic analyses, from aerodynamic, cinefluorographic and other studies a wealth of data on the peripheral mechanisms of speech is now available. EMG, however, represents a step in a central direction, a challenging tool to approach the speech motor commands of the central nervous system.

The application of EMG in speech research has been surveyed and discussed by Cooper (1965), Fromkin (1965) and Fromkin & Ladefoged (1966). EMG-studies of the palatal muscles in humans have been reported by a number of investigators.

Li & Lundervold (1958) used concentric needle electrodes and an oral approach to the palatal muscles in 5 normal and 11 cleft palate subjects. They reported on difficulties in exploring the soft palate muscles individually but they claimed that they were able "to distinguish the tensor palati from the remaining muscles." "The ap-

pearance and beha ior of the motor units registered from the tensor palati and from the remaining palatine muscle were dentical." "The duration did not exceed 3 milli seconds, average 1.6 milliseconds"

B oadbent & Swinyard (1959) used monopolar insulated needle electrodes and an ral approach in 18 cleft palate patients that had been subjected to palatopharyngeal flap operations. They recorded muscle action potentials from the flap pedicle and from other palatal muscles d ring swallowing "Similar electromyographic patterns n voltage, frequency and wav form were noted in the pharyngeal flap as well as in normal tensor palatini, levator palatini nd the superior constrictor On the basis of these studies it is felt that the pharyngeal flap is a dynamic muscular unit. These auth rs also stated that th opt cal and acoustical displays of th tensor EMG were regularly different from those of the levator whereas the levator and the constrictor were found similar n this respect.

Basmajia & Dutta (1961) used flexible bipolar needle electrodes and an oral approach in 10 normal subjects Recordings were made from the levator and the superior middle and inferior constrictors while the subject was sucking water through a straw and during swallowing They found that the levato was slightly active during suction, but the constrictors remained inactive. Data on "duration f EMG-activity" and "a erage amplitude" during swallowing were also presented.

Harris, Sch ey *et al.* (1962) used suction cup surface electrodes in a study on oral and nasal labial stops. For the nasal /m/ no activity was observed anywhere in the palatal region. For /p/ and /b/ however there is an dentical burst of activity which can be measured on either superior r inf rio palatal surface.

Fritzell (1963) presented a prel minary report on a nasal approach with concentric needle electrodes in a study of connected speech.

Hering, Hoppe *et al* (1965) used concentri needle electrodes and an oral approach in 40 cleft palate subjects. They compared the number of action potentials recorded from arious parts of the soft palate during phonation with the speech proficiency of th subject.

Iwashita (1965) used Eust chian tube catheter t design a special concentric needle which he introduced in the nasal ca ties and inserted into the superior sur face f the soft palate H assigned the activity recorded by this electrode to the levat and studied ts relationships to EMG-recordings from M. orbicularis oris and M. cricoarytenoideus lateralis during the production of Japanese speech sounds The delay between onset of EMG-acti ity and onset of audio signal was measured, as well as the " lectrical potential" in μV fo various vowels and syllables He found con iderabl ntra-subject nd inter-subject variations.

Cooper (1965) surveying EMG-research at the Haskins laboratories, described some pilot studies on the palatal muscles by means of surface electrodes. The preli m nary findings ndicated clo-e relationship between EMG-acti ity in the posterior soft palat and elopharyngeal closure A partial, but less close, relationship was found between EMG-activity in the posterior pillar and velopharyngeal closure.

Bohme S am *et al* (1966) used concentri needle electrodes and an oral approach to the tensor nd levator muscles claiming that optimal EMG-recording could not be btained transnasally They studied respiration and sustained owel sounds in 10 nor

mal subjects and found a difference in EMG-activity between the levator and the tensor muscles.

Lubker & Curtis (1966) used surface electrodes and an oral approach in a study where palatal EMG and cinefluorography were carried out simultaneously. Variations in velar position and EMG-activity were studied "during the production of sustained vowel and nasal phonemes and during alterations in duration and phonetic context". The hypothesis that the velum acts in an all-or-none (on-off) fashion was rejected. Lubker's more extensive accounts of this study will be commented upon in chapter III.

In conclusion, this review of the literature indicates that the overall EMG-activity recorded from the soft palate is closely related to the production of oral speech sounds and to velopharyngeal closure. The information on the discrete activity of the individual palatal muscles in speech is very limited, however, and the available data must be interpreted with great caution. The majority of the authors quoted have used needle electrodes and an oral approach. Besides the considerable difficulties in placing the electrodes correctly in the small tensor and levator muscles in this way, and besides the tendency of needle electrodes to migrate in a highly mobile region like this, procedures of this kind obviously interfere with articulation, which seriously limit their use in the study of speech.

No intra muscular recordings have been made from the superior constrictor, palatoglossus or palatopharyngeus muscles during speech, and the EMG-findings from the tensor are not in agreement. It is highly desirable to develop technique to make intra muscular recordings from all muscles that control velopharyngeal closure. Likewise it is very important that these recording procedures do not influence the movements of the vocal tract during speech.

II A QUALITATIVE STUDY OF THE ACTIVITY OF THE PALATAL MUSCLES BY MEANS OF ELECTROMYOGRAPHY

MATERIAL AND METHODS

The procedures used in the present investigation were developed at the University of Göteborg. To avoid interference with articulation as much as possible it was decided to insert the electrodes for the tensor, levator and superior constrictor muscles through the nasal cavities into the side walls of the epipharynx. A series of dissections on fresh human specimens were performed to study the topography of the tensor and levator muscles in relation to the orifice of the Eustachian tube. With a conventional type of concentric needle electrodes (DISA 13K53 and 13K54) recordings were made from subjects with facial defects and from normals, and the preliminary results were reported (Fritzell, 1963). Since the placement of these electrodes was very difficult and unreliable in normal subjects, another type of electrode was designed and manufactured, as described in the next section. Procedures to insert this electrode and to support it during the recordings were worked out and tested on a great number of subjects, before it was felt that the electrodes could be inserted without undue discomfort to the subjects and the muscles located with a fair degree of success.

During this long preparatory phase of the investigation, recordings were made from altogether 18 subjects with facial defects after maxillary tumour surgery and 81 normal subjects. The experimental data upon which the present report is based, were obtained at the University of Michigan, where the study was completed.

INSTRUMENTATION

An outline of the instrumentation used in this study is presented in the block diagram of Fig. 2.

Electrodes. In the present study two kinds of electrodes were used. For the *transnasal approach* to the tensor, levator and superior constrictor muscles a special type of "monopolar" needle electrode was designed (Fig. 3) similar to the electrode described by Iwashita (1965). A piano wire (steel, 0.4 mm in diam.) was cut to a length of 200 mm. One end was bent into the form of a small loop to serve as a handle. The wire was coated with 6 layers of Araldit varnish, following a procedure reported by Reichmann and Jonsson (196) to produce electric insulation.[1] The straight end of the wire was then ground at an angle of 20—30° to form a needle for insertion. One conductor of a small, shielded, two-conductor cable was soldered to the loop of piano wire. The other conductor of the cable was soldered to a small connector. A Eustachian tube silver catheter was used to guide the piano wire electrode to the side wall of the epipharynx during the insertion and to support it during the experiment. The catheter was exteriorly insulated by varnish except at the very tip. A small connector was attached to it to match the connector of the two-conductor cable. A small plastic tube of appropriate length was used to facilitate the passage of the piano wire electrode.

[1] This coating was performed by the Department of Anatomy, University of Göteborg, and the valuable help of Drs. Jonsson and Reichmann is acknowledged.

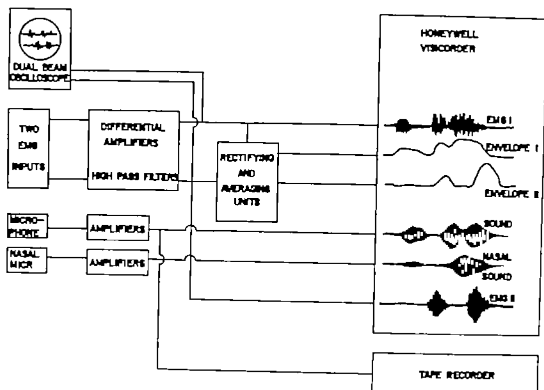


Fig 2 Block diagram of the electromyographic and sound recording equipment used in this study

through the silver catheter and to act as an extra insulation. Electromyograms obtained with this electrode represent the voltage between the tip of the piano wire in the muscle and the tip of the silver catheter in contact with the mucous membrane. The area of the uncoated tip of the piano wire was calculated to be approximately 0 6 mm²

For the *transoral approach* to the muscles of the pillars of the fauces a very fine flexible bipolar wire electrode was used (Fig. 4) a type of electrode described by Basmajian and Stecko (1962) Two pieces of wire (Karma alloy wire polyurethane enameled, 0 05 mm in diameter[2]) were cut to a length of 200 mm and passed through a short hypodermic needle of small size Swedish gauge 20. The polyurethane insulation was burned off at the ends of the wires leaving approximately 1 mm bare at the needle tip where the wires bent to produce two hooks. The hypodermic needle was used to insert the wires. When it was withdrawn the hooks held the wires in the muscle After the experiment a gentle pull caused the hooks to straighten out so that the wires were released Electromyograms obtained with this bipolar electrode represent the voltage between the tips of the two wires. The area of the uncoated tip of one wire was

[2]) manufactured by Driver Harris Corp., Harrison, New Jersey U.S.A supplied to the author by Dr B. Jonsson, Department of Anatomy University of Goteborg.



Fig. 3. Eustachian tube catheters and piano ire electrode Note the different degrees of cur ature of the catheters the pper catheter is used for insertions into the levator and superior constrictor muscles the lower catheter bent at an angle of 90° is ed for the insertion into the tensor muscle. The piano wire electrode is show th fine plastic tubing covering the hole length except for the ery tip. During the passage through the catheter the plastic tubing covers and protects the sharpened tip of the pian ire.

calculated to be appro imately 0.16 mm² The electrodes were sterilized before usage by exposure to ethylene oxide gas for 6 hours at a temperature of 66 C (118° F)

Ampl f rs. A modified H neywell Electronic Medical System was used. Each EMG channel had differential vacuum tube amplifier driv g a differential transistor amplif er The RC coupl g network between stages in the acuum tube amplif ers was selected to provide a low frequency cut-off t 40 Hz (12-dB-per-octa e rejection) Each input unit had a calibration oscillator providing signal of 600 μV peak to peak. The amplification f the EMG-signals was kept constant d ri g th experiments described this hapter It was set to gi e display of 25 mm for the 0.6 mV calibrating signal in both channels.

Oscilloscope — Mon tor A Du Mont, type 322 dual-beam cathode-ray oscillograph was used as a mo itor

Rectify g and aeragi g ci cuits Tw identical electronic circuits were bu'lt t provide rectified and eraged envelope display of the muscle action potentials. (Electric circuit d agram, F g. 5) The time constant of the RC filters was approximately 25 msec.



Fig. 4. The thin wire electrodes passed through the hypodermic needle which was used to insert them into the palatoglossus and palatopharyngeus muscles. The uncoated tips at the hooked ends of the wires are seen. (Magnification 3.4 x)

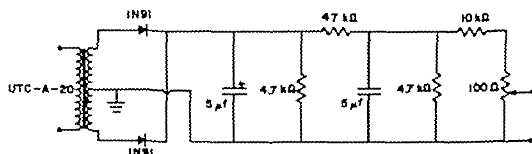


Fig. 5. ... diagram of the ...



Fig. 6. Left A close-up of subject showing the adjustable glasses and levers supporting the Eustachian tube catheters and piano wire electrodes.
Right An experimental subject seated in the comfortable chair in the recording room. The position of the microphone is shown, and the Oscilloscope-Monitor is seen through the window

Recorder The amplified muscle action potentials were recorded on a Honeywell 1108 Visicorder Oscillograph The Visicorder provides a direct record on light-sensitive paper The tracings are written by miniature galvanometers with mirrors deflecting the high intensity ultraviolet light from mercury vapor lamp onto the moving paper The records are automatically developed by a photolizing process within a few seconds when exposed to fluorescent light or sunlight. The frequency response of the galvanometers used for the direct EMG-records was flat up to 1800 Hz. The rectified signal from the mean voltage circuits were fed into galvanometers with flat response up t 24 Hz. A paper speed of 50 mm per second was used this study Vertical time lines were produced on the record, at a frequency of 1 per second. The recording paper was Kodak Linagraph Direct Print Paper type 1813.

Sound Recording Equipment

Microphones. Three types of microphones were used. For conventional recording of the speech signals a Sennheiser MD 3 dynamic, velocity type microphone was used. It was positioned close to the right angle of the mouth as seen in Fig. 6. For the recording of swallowing sounds a Sennheiser MM 51 magnetic throat microphone was used, placed on the side of the neck, lateral to the larynx. (Logan *et al.*, 1967) These two Sennheiser microphones were used alternatingly with the same amplifiers In few subjects recordings were also made with small microphone inside the nasal cavities For this purpose Knowles miniature magnetic microphone BJ 1590 was used.[1] Its size was 1×5.5×7.8 mm. A thin shaft of aluminum, 15 mm long was glued to the microphone The shaft enabled the experimenter to place the microphone in the nasal cavity Thin plastic covers were used around the nasal microphone to prevent the spread of infections

[1] The use of this miniature microphone inside the nasal cavities as an indicator of nasal resonance was suggested by Dr. Franklin Cooper Haskins Laboratories New York

Amplifiers With the Sennheiser microphones an audio amplifier Am-1115/UNS-1 manufactured by Harvey Wells Electronics, Inc., Southbridge Massachusetts U.S.A was used

For the nasal microphone a small transistor audio amplifier was built, capable of amplifying the input signal 9 times.

From these audio amplifiers the signals were fed into a Honeywell Model T6GA Galvanometer amplifier

Recorders The microphone signals were recorded on the Visicorder simultaneously with the EMG signal The signals from the mouth and throat microphones were recorded by means of a galvanometer with a flat frequency response up to 1800 Hz. The nasal microphone signal was recorded by a galvanometer with a linear frequency response up to 1000 Hz.

The speech signals from the mouth microphone were also magnetically recorded on a Channel Master model 6130 tape recorder for auditory playback and correlation with the visual record from the visicorder

MATERIAL

The experimental subjects were 26 University of Michigan students, who had volunteered for the study Only subjects who still had their tonsils were accepted There were 14 males and 12 females, in ages ranging from 17 to 30 years. They originated from various parts of the U.S.A and did not constitute a homogenous dialect group.

PROCEDURES

Recording room The subjects were seated in a comfortable chair with head rest in a shielded and sound-treated room (Industrial Acoustics, model No. 60) (Fig 6) The recording apparatus was located outside this room. A window and an intercommunication system allowed for observation and instructions from the experimenter who was also outside the room, operating the recording equipment during the experiment.

Topical anesthesia To avoid pain during the placement of the electrodes and during the experiment, the mucous membranes of the nasal cavities (along the inferior turbinates) and the epipharyngeal walls were painted with a solution of 5 % cocain HCl Applicators shaped like Eustachian tube catheters were used to permit painting of the area below the tubal orifice

Placement of the electrodes From dissections it has been established that the levator muscle was to be found immediately under the mucous membrane in an area below and behind the Eustachian tube orifice, and that the tensor was to be found somewhat deeper (more lateral) in an area posterior to the medial pterygoid plate anterior to the levator and below the tube orifice (Fig 7) To direct the piano wire electrodes to these two areas of insertion, Eustachian tube catheters of different degrees of bending were used The catheters aimed at the tensor had an angle of 90° and those aimed at the levator were bent at an angle of 120—135 (Fig 3)

The first step in the placement of the tensor and levator electrodes was to pass the catheter through the nasal cavity placing its inner end in the orifice of the Eustachian tube This position was checked by inflation of air through the tube into the middle ear and by listening to the noise produced through a rubber tube connecting the ear

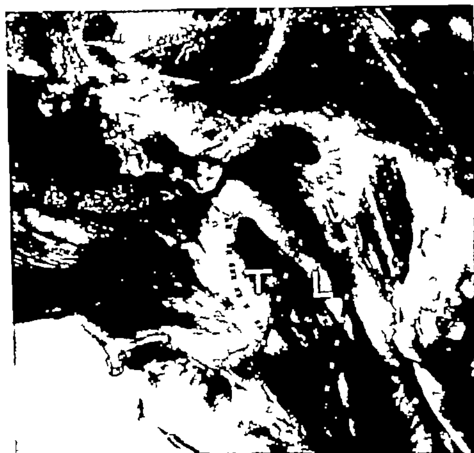


Fig. 7 The right lateral wall of the epipharynx as seen on specimen before the dissection. At the top the base of the skull is shown, and at the bottom part of the soft palate T the left are the nasal conchae and to the right the back all of the epipharynx. In the middle of the photo the orifice of the Eustachian tube is seen. The tensor of the soft palat can be found under the area T and the levator under the area L. (This figure is reproduced ith the permission of the editor of Folia Phoniatrica, in which journal it as published in Vol. 15, 1963.)

canal f the subject to the ear canal f the experimenter (The deta ls of this routine procedure are described in te tbooks f otorhinolaryngology) During this inflation the curved end f th catheter was d rected cranially and laterally t an angle of approxi mately 15 t the ertical plane Following this control of the position the catheter was rotated laterally that the curved end of the catheter had a horizontal position and the electrode would hit a point below the orifice of the Eustachian tube The catheter was held in this position duri g the xperiment by a pai of adjustable eyeglasses with levers and screws (Fig 6) The electrodes were inserted in small steps under i ual observa tion f the monitori g oscilloscope and the subjects wer asked to phonate and to swallow Recordings from th levator w re obta ned with depth f electrod insertion f 1—2 mm, while recordings from the tensor required an insertion f 4—5 mm. Fo the superior const ictor muscle a "levator-type" catheter was used. The catheter was passed through the nasal cavity to th back wall f the pharyn and its interio end

elevated to provide an insertion of the electrode in the back wall at the estimated level of velopharyngeal closure. When a good recording from the constrictor was not obtained by this procedure the catheter was somewhat drawn back and rotated laterally to provide an electrode insertion in the side wall of the epipharynx posterior to the salpingo-pharyngeal fold. Ordinary mirror-epipharyngoscopy was made to check the position of the catheters in relation to the Eustachian tube orifice and the salpingo-pharyngeal fold. In sensitive subjects, gagging reflexes prevented the execution of this examination, however, and this check could not be carried out in all subjects. A successful examination of every subject might have been achieved by anesthetizing the mucous membranes of the throat, but because minimal interference with the vocal tract was desirable this was not done.

The hypodermic needles with the fine wire electrodes used for recordings from the palatoglossus and palatopharyngeus muscles were passed through the mouth and inserted into the anterior and posterior pillar of the fauces respectively by means of a pair of Blakesley forceps for ethmoid surgery. The placement was made such that the uncoated tips of the wires became located 1—2 mm under the mucous membrane 5—10 mm from the point of insertion (Fig. 8). No topical anesthesia was used in this area.

The electrodes were inserted on the right or left side at random. It was felt that there was no difference between recordings from right and left, but this matter was not studied systematically.

The placement of the electrodes was often difficult, and a satisfactory EMG-recording was by no means always obtained. The over-all rate of success was 69 %. In some subjects with deviations and bony cristas of the septum a catheter could not be passed through the nasal cavity on one side. In some the dimensions of the epipharynx were such that the tensor catheter could not be rotated and properly positioned. In other subjects the catheters were properly placed as described above and still no satisfactory EMG-recordings from the levator and constrictor muscles could be obtained in spite of 2 or 3 repositionings of the electrode and attempts to locate each muscle. The rate of success was higher with the palatoglossus and palatopharyngeus muscles, most probably because the point of insertion could be observed visually. However, the wire electrodes sometimes did not stay in the muscles but were pulled out by swallowing or other movements. Usually no more than 3 or 4 attempts were made to find a muscle and to obtain a good recording from it. The time limit for the whole experiment was set at 90 minutes.

A certain degree of discomfort was experienced by all subjects but there was a great deal of variation in this respect. For many the initial painting of the mucous membranes of the nose and epipharynx with the anesthetic solution was the most unpleasant part of the experiment. The passing of the catheter through the nose and the manipulation with the epipharyngeal wall was more disagreeable than the transoral approach to the pillars. A slight pain was sometimes experienced during the insertion of the piano wire electrodes into the epipharyngeal walls. A few subjects experienced discomfort from the catheter and piano wire during swallowing but nobody reported discomfort or pain during speech. The fine wire electrodes in the pillars were usually not felt at all by the subjects, except at the angle of the mouth. Most of the subjects tolerated

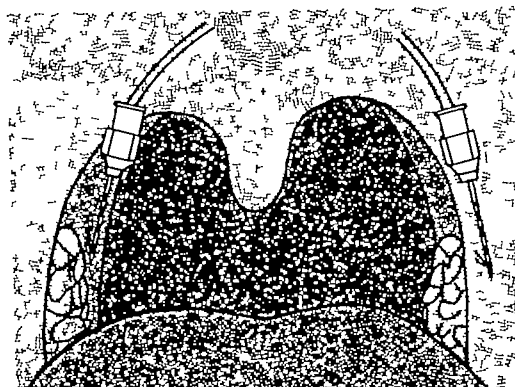


Fig. 8. A schematic drawing of the insertion of the hypodermic needles and the thin wire electrodes into the pillars of the fauces. The one on the reader's right is aimed at the palatoglossus muscle, and the one on his left at the palatopharyngeus.

the manipulations and electrode insertions very well and did not seem to mind at all. During the past two years the experimental subjects have routinely been asked after completion of the experiment whether they would come back for another session of the same kind. Out of some 60 subjects asked only 3 did not wish to do so.

The recording program. Various speech samples as well as other activities related to velopharyngeal closure were recorded. All subjects carried out the whole program.

1. Repeat the test sentences
   "This is a foolish theory."
   "Marilyn sneezed during every meal."
   "It do not think so."
2. Read two lists of 18 VCV (vowel-consonant-vowel) utterances.
3. Whisper say about "Mummy" "daddy".
4. Breathing — with an open mouth — through the nose, through the mouth, and through the nose.
5. Oropharyngeal inflation with leak. Oral inflation with nose breathing.
6. Swallow — whistle — blow — swallow
   Suck water — swallow
   Swallow — /a/ — swallow — / / — swallow
   Chew a cracker — swallow

The VCV utterances consisted of the neutral vowel /ə/ followed by a voiced plosive sound /b/ /d/ /g/ or a nasal sound, /m/ /n/ /ŋ/ and ending with a stressed vowel /a/ /i/ or /u/. The total number of possible combinations is 18. Two lists of these were made with the order of utterances reversed.

The speech samples were primarily selected to demonstrate the differences in the production of oral and nasal sounds. The first sentence was composed by oral sounds only with a number of voiceless fricatives, for which a competent velopharyngeal closure appears to be particularly important. In the second and third sentence there were both oral and nasal sounds, the second sentence beginning with a nasal consonant. The VCV utterances were chosen to demonstrate the difference between voiced oral plosives and their corresponding nasal sounds, and the difference between low and high vowels.

Instruction of the subjects. Before the experiment the subject was given a short period of training in the performance of the program. During the recording an assistant gave the instructions for the various items in the recording program to the subject one by one. A signal was given to tell the experimenter to start the recorder and following this a signal was given to the subject to go ahead with his performance of the assigned task.

Examination of the records. In this part of the investigation, which is of a descriptive qualitative type, the records were judged mainly by visual inspection. In the case of the VCV utterances the rectified and averaged display of the electromyograms were also traced and superimposed as described by Cooper (1965) and Fromkin (1965) in order to somewhat "counteract" the random variation of the EMG curves and facilitate the evaluation.

RESULTS

The figures 9—14 and 18—23 are representative examples of the electromyographic records. The symbols of the international phonetic alphabet have been used to indicate the nature of the speech sample and the symbols have been lined up with the corresponding events in the microphone tracings. The phonetic transcription represents what the subject was supposed to say during the recording. Dialectal variations and omissions of sounds have not been considered. The phonetic text thus represent the target speech sample rather than a close transcription of what was actually said.

The duration of each record, from the onset to the termination of the tracings, has been given in the legend.

GENERAL FINDINGS

As mentioned, the overall rate of successful placement of the electrodes was 69 %. Consequently when the time limit set for the experiment allowed for attempts to record from all 5 muscles of one subject, satisfactory recordings were usually obtained only from 3 or 4 of these. The superior constrictor was more difficult to locate properly than any of the other. Out of 19 attempts to record from this muscle only 10 were successful. In the following the [illegible] report on the findings for any one muscle is based upon at least 10 [illegible] different subjects.

With few exceptions the electromyograms showed absence of activity all the muscles during rest and quiet breathing. In speech and in the other activities studied, there was a varying degree of EMG-activity. The *onset, presence and termination* of activity varied between muscles, and more or less typical overall patterns of behavior could be distinguished.

The amplitude of the EMG varied considerably from one recording to another. Thus, measured from peak to peak, the maximum amplitude *of the interference* pattern records obtained from 18 levator muscles in different subjects during speech ranged from 0.3 mV to 2.5 mV with a mean of 1 mV. An amplitude *ariation of the* same order was recorded fo the arious muscles within one subject. Most probably these inter individual and intra-ind dual voltage differences re caused by *riations* in the electrode placement, and they can not be held to reflect any real differences. A high ampl tude signal primarily indicates that the electrode is located close *to acti* motor units, whereas a low amplitude ind cates recording at a certain distance from active motor units. This nterpretation of the voltage variations is supported by the findings from repeated recordings in the same subjects at different occasions. During the recording from one muscle however the maximum amplitude usually aried within small limits and only in a few isolated records were obv ous changes noted, indicat ng that a definit change in the position of the electrode had taken place

SPEECH

Sentences In the recordings f th sentences which were repeated by the subjects, the acti ity of the muscles during connected speech was displayed.

In the first of these sentences "This is a fool sh theory" (Fig 9) made up of oral speech sounds only the *levator* howed continuous ctivity The onset of this activity preceded the onset of sound The t rmination of the levat acti ity occurred slightly before or simultaneously with the termination of phonation. The degree f acti ity showed fluctuations during the performance In some cases ncreased activity occurred with the stressed syllables of the sentence, in others the peaks of activ ty seemed to be primarily related *to the prod ction of the* fricative sounds During the production of "Marilyn sneezed during every meal" (Fig 10) the levator demonstrated bursts of action potentials alt rnating *with periods f n* or very *little* activity these latter periods bei g closely related to the production of nasal sounds. Th lev tor acti ity alw ys preceded the onset of the initial */m/ but the degree f this* initial cti ity was low The / / this sentence surrounded by nasal sounds was in ariably preceded by a high peak of levator acti ity In "I do not th nk so" (Figs 11 and 12) the levato typically howed 3 periods f activity related to the 3 groups f oral sounds in this sentence This on and ff pattern f the levator EMG in relation *to th oral and* nasal sounds always preceded the corresponding event in the acousti recording. The characteristics of the levator acti ity described abov were ery consistent and occurred i all record ngs from this muscle

The activity f the *tensor* during connected speech varied considerably (Figs 13 and 14) I out f the I recordings from this muscle there was very little activity r none at all. I those nstances where a fai degree f acti ity was recorded, there was little *greement between subjects* Apart from the onset of acti ity before the onset

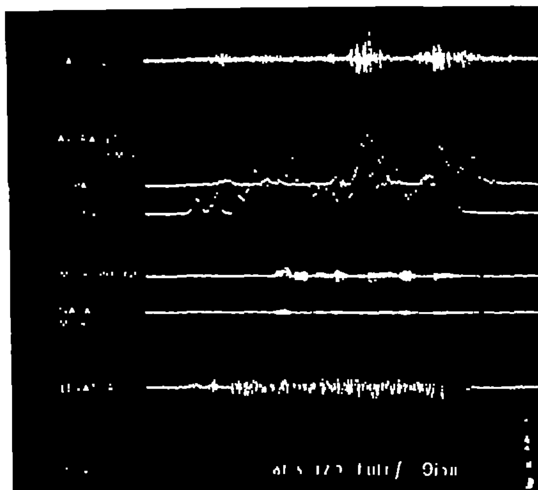


Fig. 9 A recording f the sentence "This is foolish theory The acti ity of the lev tor starts ll before the onset f the microphone signal conti ues throughout the utterance d ends ith the end of phonation. The palatoglossus is initially weakly acti ith period of ilence With the transition from /ʃ/ to / / there is burst of cti ity and lso t the end f phon tion There is ery l ttle response i the nasal microphone — D ration of the recordi g show 4 l s.

of sound and a peak of activity in the beginning of th sentences no con istent mode of "beha ior" wa found.

The sentence recordings from the *superior constrictor* were similar to those of the levator (Fig 12) The constrictor was acti luring the production of oral sounds and t acti ty wa weak or absent during the production of nasal sounds. The onset of /s/ n "Marilyn neezed " wa always accompanied b a burst of potential of high amplitude

The *palat glossu* muscle showed weak acti ty or wa completely ilent in the beginning of "Th is a fool h theory" (F g 9) but it demonstrated strong burst of potential ding wit the tran t on from /ʃ/ t /a/ in "fool sh and with the t rmi nati of the se ten I M r lyn neezed dur ng every meal" the palatoglossus was

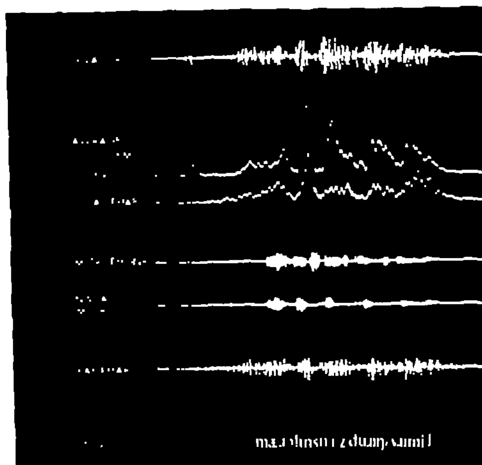


Fig. 10. A recording of the sentence "Marilyn sneezed during every meal". The levator muscle is active before the onset of phonation, but only to a moderate degree. Alternating periods of high and low activity follow related to the production of oral and nasal speech sounds, respectively. The highest peak is for the /i/ between the two nasal sounds. The palatopharyngeus activity is apparently synchronous with that of the levator; the only major difference seems to be that /i:/ shows a peak in the averaged levator EMG, but no corresponding peak in the palatopharyngeus envelope. — Duration 3.3 s.

inactive or weakly active with the onset of the sentence, but demonstrated bursts of potentials preceding the nasal sounds within the sentence and at the termination of phonation. During the production of oral sounds the activity was weak or absent. Likewise in "I do not think so" (Figs. 11 and 14) the palatoglossus was active in the beginning, for the nasal sounds and at the end.

The recordings from the *palatopharyngeus muscle* (Figs. 10 and 13) during connected speech showed a great deal of variation. In all subjects there was some activity but often of a low degree. In those where a more active participation was noted the activity appeared to be related mostly to oral sounds, and bursts of potentials often occurred with the onset of / / nasal sound within sentence were usually preceded by a decrease of activity. These features however were not as clear and consistent as were

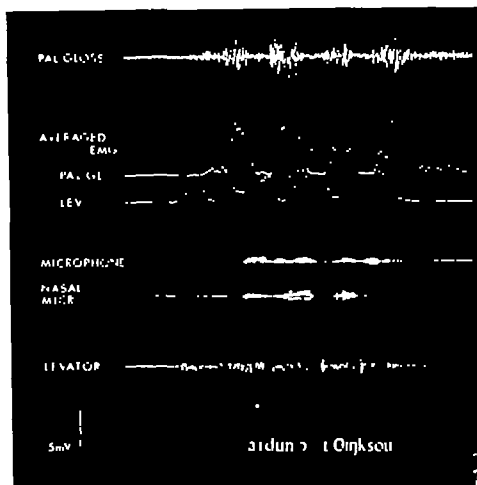


Fig 11 A ecording of the sentence I do not think so The lev tor shows three periods of activity during phonation, related t the three groups of oral sounds. The palatoglossus shows four *burst of spikes at the onset f phonation, before the production of the t nasal sou ds* and at the end f phonation. The alternating acti ity is learly demonstrated i the t en elopes, the lev tor relaxi g hen the palatoglossus is contracting, nd ice ersa. — Duration 3.4

those of the levator In some cases the palatopharyngeus like the palatoglossus wa markedly active at the termination of phonation.

VCV -utterances The VCV utterances were selected to demon trate differences in the production of oral and nasal sounds and in th production of high and low vowels These nonsense word were read by the ubjects from lists during the experiment. Two list were read each time the program wa recorded. The same muscle was often recorded from 2 or 3 times usually the levator in combination with various other muscles and thus 4 or 6 VCV record were produced with the electrode in the same position.

Many ubject proved to hav difficulties in producing th /ŋ words /aŋa/ /aŋi/ and /aŋu/ properly in p t of the rehearsal preceding the experiment. Most of them

4 t ... ... pd ... so

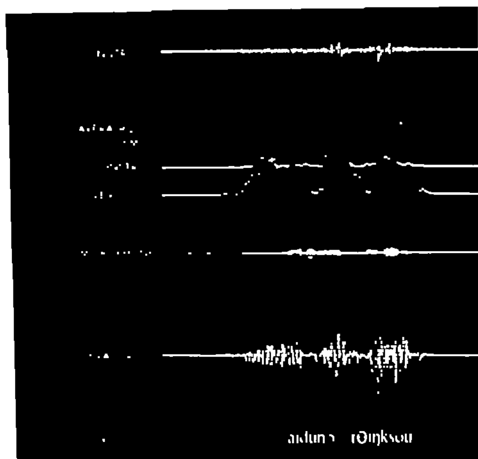


Fig. 12. A recording of the sentence "I do not think so". The levator shows the same pattern as described for fig. 11; the constrictor likewise shows three bursts of activity synchronous with the levator bursts, but with far fewer spikes. — Duration 3.3 s.

tended to insert the plosive sound /g/ between the /ŋ/ and the final vowel. Therefore these /ŋ/ words were for the most part disregarded in the subsequent study of the data.

In the evaluation of the recordings from these VCV-utterances, attention was primarily directed towards the rectified and averaged displays of the electromyograms. These envelopes were traced on tracing paper and superimposed (Fig. 15). The account given below is mainly based upon the inspection of these superimposed envelopes. In a few selected cases the height of the envelopes was measured at 40-msec intervals and averaged to produce a single curve representing 10 or more envelopes (Figs. 16, 17). This is a time-consuming procedure which, as suggested by Fromkin (1965) and others, should preferably be carried out with the aid of a computer

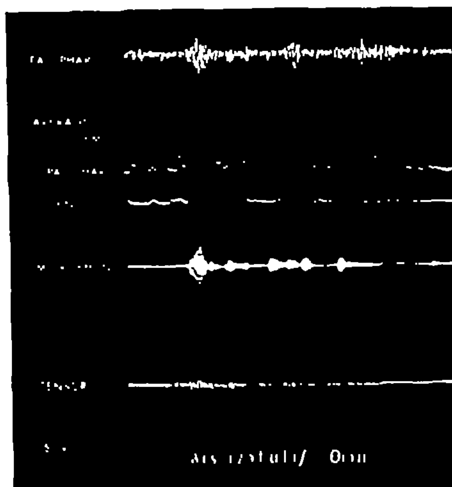


Fig 13. A recordi g of the sentence "Thi I foolish the ry The palatopharyngeus is not continuously acti with the levator but bet een short periods f silence it sh burst of rying degrees of cti ity The strongest f these burst seem to be rel ted to the /s/- d /f/-sou ds and to the end f phonation. The tensor show activity in few motor it mai ly duri g t periods losely related t the trong burst of the palatopharyngeus. — Duration 3.. s.

measuring at shorter interval from tape recorded electromyograms and a eraging o er at least 20 samples of ea h utterance

The activity of the *levator* was considerably high r in the oral consonant utterances than in those with a nasal consonant (Figs. 15 16, 18) Thi was a con istent finding When the three final vowels were compared, different degrees of levator activity were also noted th most common finding being an increase from /a over /i/ to /u/ These d fferences wer mor marked and more often f und in th nasal consonant utterances than in the ral ones. No d ff rence cou l be distingui hed between the t ree different voiced top soun l except i a ingle subject who showed a "dip" of the envelope correspond ng to the production of t /g/-sound No difference was found between the /m/ and / /-soun l productions.

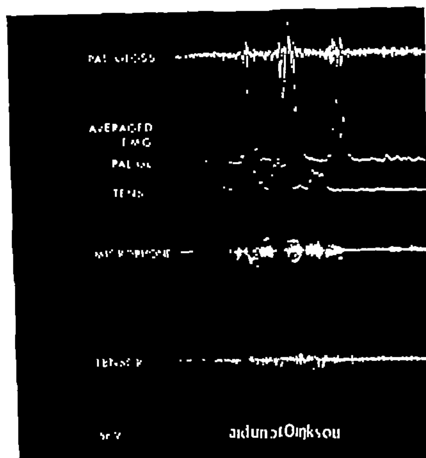


Fig. 14. A recording of the sentence "I do not think so". The palatoglossus characteristically shows some activity before the onset of phonation and then three bursts of spikes related to the nasal sounds and the end of phonation (compare fig. 11). The tensor here shows 3 periods of activity, the first almost simultaneous with the palatoglossus burst, but the two following ones more and more out of phase. — Duration 2.6 s.

In 9 subjects out of 12 the *tensor* showed none or very little activity during the VCV utterances. In those three cases where the envelopes were of a magnitude that allowed for tracings to be made there was little agreement between subjects.

The activity of the *superior constrictor* was similar to that of the levator. There was more activity during the production of oral than nasal utterances. Likewise, the activity during the production of the final vowel tended to increase in the series /a/ — /i/ — /u/.

The *palatoglossus* muscle usually showed slight activity during the production of the first vowel /a/. Often the onset of this activity preceded the onset of sound. In all subjects the muscle was completely inactive or showed a very low degree of activity corresponding to the production of the /b/- and /d/-sounds, whereas a burst of pala

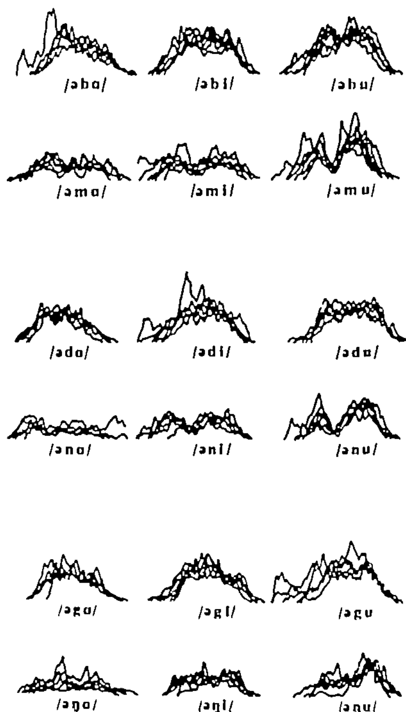


Fig. 1. Superimposed trac[in]gs of the [ve]ctor en[ve]lope from 5 series [o]f VCV-utterances by subject [re]ading [a]t rather high [s]peed. The t[r]acings f[or] one [u]tter[ance] has [a]ll been lined up at the same poi[n]t, [i.e.] the onset [o]f the second [vow]el [in] the [m]icrophone record. It [can] be seen that there is more [reg]ul[a]rity [in] the oral t[racings] than [in] the nasal ones. If comparisons [a]re made horizontally [a] consistent [i]ncrease in the [he]ight [o]f the envelope [is] see[n] for the final [v]owel, from /a/ t[o] /i/ t[o] /u/. Th[is] tendency [is] he[re] for [a]ll consonant sounds, but more marked for nasal utterances. Comparisons made [v]ert[ica]lly do not show [an]y differences except for the oral-nasal distinction: there is no essenti[a]l diff[er]en[ce] bet[w]een the en[v]elopes for /[a]ba/, /ada/ and /[a]ga/ etc.

LEVATOR

PALATOGLOSSUS

/ P /   / P /

/ P I /   / P I /

/ P /   / P /

/ N /   / N a /

/ N I /   /aN I /

/ N /   / N /

Fig. 16. Averaged envelopes from [illegible] subject [illegible] participated in the experiment [illegible] interval of 5 months. The solid levator envelope [illegible] the average of [illegible] envelopes from the f[illegible] recording, the broken levator envelope represent[s] 21 envelopes from the second recording. The solid palatoglossus envelope is the average of [illegible] envelopes from the first recording, the broken palatoglossus envelope represents 12 envelopes from the second recording. The height [illegible] the envelopes in the illustration has been adjusted to facilitate comparison of the shapes of the [illegible] from the tw[o] recordings. As far as the levator [is] concerned, "P" in /P/ etc. stands for "plosive" [illegible] this averaged curve is composed of all /aba/, /ada/ and /aga/. "N" in /Na/ stands for "nasal" [illegible] /m/ + /n/ + /ŋ/. For the palatoglossus the same [illegible] applies, [illegible] for the fact that [illegible] utterances [illegible] dorsovelar sounds /g/ and /ŋ/ have been excluded, since they [illegible] different. The arrows under the base lines indicate the onset and end of phonation. The thin vertical lines indicate the onset of the stressed second vowel, [illegible] used in lining up the envelopes before averaging.

The shape of the averaged envelopes from the [illegible] recording [illegible] similar. The difference in shape determined by the final [illegible] readily appreciated. The difference between oral and nasal utterances is [illegible] in the levator envelopes, but does not seem to be present in the palatoglossus curves. The difference between the levator and the palatoglossus envelopes is quite clear.

PALATOGLOSSUS

/aba/ama/ /ada/ana/ /aga/aŋa/

/abi/ami/ /adi/ani/ /agi/aŋi/

/abu/amu/ /adu/anu/ /agu/aŋu/

Fig. 1 Averaged envelopes from the palatoglossus muscle in one subject. Each curve represents an average of 5 utterances. The solid lines represent the oral utterances and the broken lines the nasal utterances. Arrows indicate average onset and end of phonation. Vertical lines the onset of the final vowel. The difference between oral and nasal utterances is marked. The peak of activity corresponding to the dorsovelar consonant can be seen and it is also well demonstrated that the production of /ŋ/ is associated with higher palatoglossus activity than /g/. These palatoglossus envelopes are markedly different from those in fig. 16.

toglossus potential preceded the /g/-sound. Likewise there was always a marked activity related to the /ŋ/ sound and this activity was more pronounced than the /g/ activity in 12 of the 16 cases. There was a great deal of variation between subjects in palatoglossus activity during the production of the final vowel: there seemed to be no consistent, systematic relationship (compare Fig. 16 and Fig. 17). Most subjects demonstrated a marked activity at the termination of phonation, and this activity was usually higher for /u/ than for /a/ and /i/.

The *palatopharyngeus* recordings from the VCV utterances showed no appreciable difference between the oral consonant sequences and the nasal consonant sequences. There was none or very little activity related to the oral plosive consonants. The palatopharyngeus participation in the formation of vowels was of a higher degree for /a/ than for /i/ and /u/ (Fig. 18).

Whisper, conversation, shouting. To give an indication of the relationship between palatal EMG and intensity the subjects were asked to say mummy and

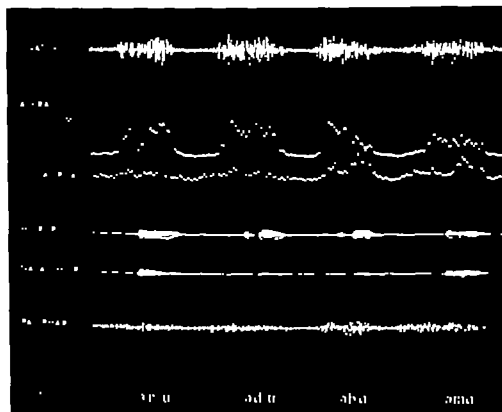


Fig 18. A recording of 4 VCV utterances, where the difference between levator and palatopharyngeus activity is demonstrated. The levator envelopes show clear difference between oral and nasal utterances, and the levator is more active for /u/ than for /a/. The palatopharyngeus envelopes show very small difference between oral and nasal utterances, and the palatopharyngeus is more active for /a/ than for /u/. — Duration 4.6 s.

daddy three times each, with whispered voice, conversational voice and shouting, respectively.

In about a third of the recordings there was no noticeable difference in the degree of EMG-activity for the three intensities. In the other two thirds the activity during shouting was more marked than during whisper and conversation. As to these latter, sometimes there was more activity during whispering than conversation, sometimes it was just the opposite, and often the degree of activity was the same for the two.

So far as these intensity relationships are concerned, there were no obvious differences between the various muscles.

NON-SPEECH ACTIVITIES

Nasal versus oral breathing. The subjects were asked to hold the mouth open and to breathe through the nose, then through the mouth, and finally through the nose again. The levator was consistently inactive during nasal breathing and active during oral

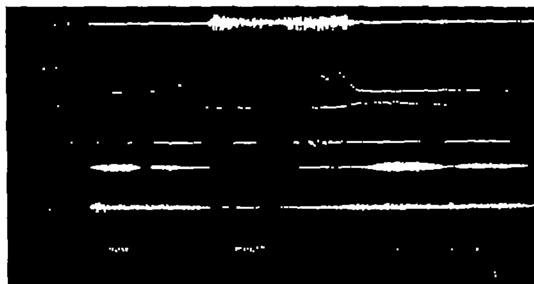


Fig. 19. A recording of breathing with the mouth open, first through the nose, then through the mouth, and finally through the nose again. The alternating activities in the palatoglossus and levator muscles are demonstrated. — Duration 8 s.

breathing (Fig. 19). The tensor usually showed weak activity or none at all. The constrictor recordings were of the same type as those of the levator. The palatoglossus demonstrated a pattern exactly opposite to that of the levator and constrictor muscles (Fig. 19). In almost all subjects the palatoglossus was more active for nasal breathing than for any other item on the recording program. During oral breathing there were few or no action potentials. The palatopharyngeus muscle showed very little difference between nasal and oral breathing; most often it was weakly active for both.

Oropharyngeal inflation. The subjects were asked to puff out their cheeks and to let some air leak out between their lips. The levator and constrictor muscles were very active during this maneuver. This was also often the case with the tensor and palatopharyngeus muscles, whereas the palatoglossus activity varied a great deal from subject to subject.

Oral inflation with nasal breathing. The subjects were asked to puff out their cheeks and breath through the nose. Typically the levator showed marked activity in the initial phase and then silence and so did the constrictor; the palatoglossus started at the end of the initial phase and was maximally active through the nasal breathing period. The tensor and the palatopharyngeus often showed mild to moderate activity throughout the two phases.

Whistle and blowing. The subjects were asked to whistle and then simply to blow some air between the lips. With very few exceptions, the EMG-recordings showed no difference between these two activities. The levator and constrictor (Fig. 20) were both active to the same degree as for oral speech sounds. The tensor was also often active, whereas the palatoglossus and palatopharyngeus (Fig. 21) recordings were inconsistent.

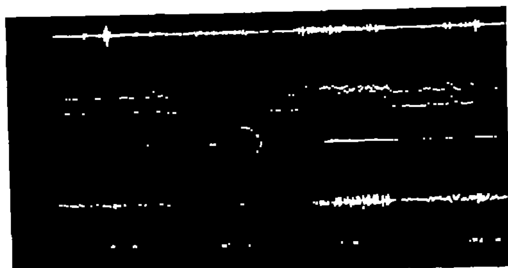


Fig. 20 A recording of swallowing, whistling and blowing, where the synchronous activity of the levator and superior constrictor muscles is shown. — Duration 8.4

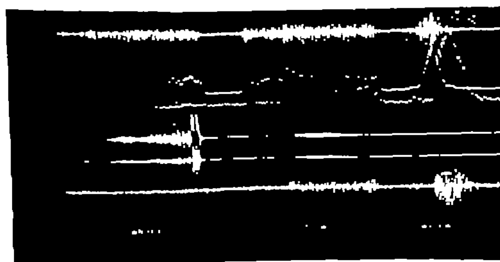


Fig. 21 A recording of whistling, blowing and swallowing, where the difference between levator and palatopharyngeus activity on blowing is demonstrated. The palatopharyngeus activity is somewhat later and is of longer duration. It can also be seen that the palatopharyngeus is more active for blowing than for whistling in this subject. Duration 8.7

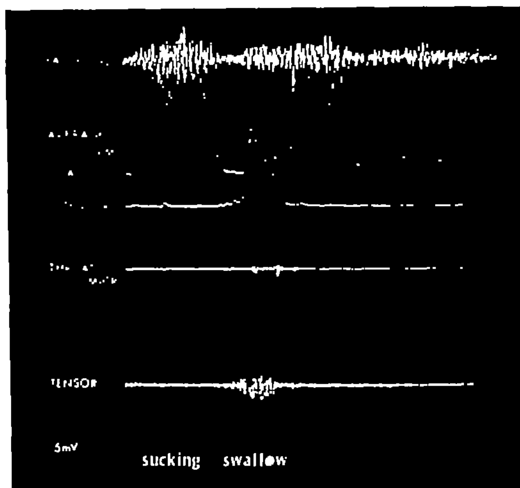


Fig. — A recordi g of sucking w ter through stra nd llowing. The palatoglossus ontraction in sucki g ry strong, llow g less so. The tensor is only acti in llowing. The throat microphon recordi g of the sounds of llowing i seen. The palatoglossus is typically ctiv fter the essation f ontraction of the other muscles i llowi g. — Duration 3.9 s.

Sucking. When the sul ject ther ucking water through a straw the palatoglossus wa alway activ the ther muscl s infrequently so (Fig. 22) Th us in 16 recordings fr m the levator there wa moderat activity in 1 ul ject only weak activity in 1 and no activity in the other 11

Chewing. Th chewing of a racker wa regularly accompanied by rhythmic contract n f the tensor and palat glossus muscles (Fig 23) The participation of the other muscles was irregular and varied between sul ject

Swallowing. Various recordings of wallowing w re obtained most of them upon instructions t th subject t wall w but also following the sucking of water through a straw and aft r the hewing of cracker Sometimes pontaneous swallowings of sali a w re lso recorded between r following speech samples. During most of these recordings a throat m rophon wa used, nd an acoustic representation of the wallow ng it w bt ned

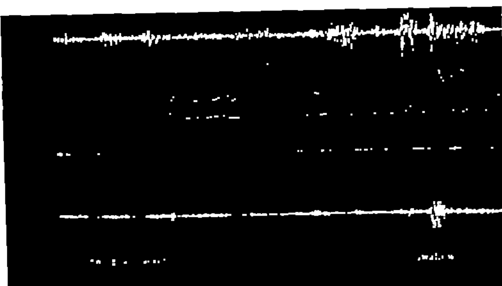


Fig. 23. A recording of chewing a cracker and swallowing. Rhythmic activities in both palatoglossus and tensor are seen. During swallowing the tensor shows its usual strong burst of short duration, whereas the palatoglossus shows varying degree of activity over a much longer period of time. — Duration 7.9

Consistently all five muscles were highly active during swallowing (Figs. 20—23). The intensity of the tensor and the palatopharyngeus contractions was always much stronger in swallowing than in any other activity. The levator and the constrictor often showed a slightly or moderately increased activity in swallowing, as compared to speech, but this was not always so. As a rule, the palatoglossus was less active in swallowing than in nasal breathing.

So far as the time relationships are concerned, the levator, the tensor and the constrictor were active simultaneously; minor variations occurred, but these were not consistent. The palatopharyngeus activity in swallowing always appeared somewhat later (Fig. 21). In 7 subjects in whom the levator and palatopharyngeus muscles were recorded simultaneously the average time delay from maximal levator activity to maximal palatopharyngeus activity was 300 msec (range 225—150 msec between subjects). The palatoglossus activity was more variable. Often two periods of activity occurred during swallowing, one preceding and one following the levator contraction; sometimes there were three activity periods, the middle one coinciding with that of the levator.

The duration of EMG-activity during swallowing showed moderate variations also within subjects. As a rule, however, the levator, the tensor and the constrictor showed one relatively short burst of high intensity, the palatopharyngeus likewise one burst of high intensity but of longer duration, while the palatoglossus was active over a fairly long period of time showing moderate intensity with fluctuations.

The swallowing sound picked up by the throat microphone [illegible] series of [illegible] occurred at [illegible] variable time after the levator contractions. The time delay measured

from the peak of the levator activity t the loudest click, was usually of the order of 200—300 msec and frequently the clicks coincided with the contraction of the pala topharyngeus muscle Inspection of the microphone tracings and listening to the tape recordings showed that the click sound of wallowing were louder for the water than for the cracker or saliva

DISCUSSION AND CONCLUSIONS

In the evaluation of the descriptive data presented in this study the first and most important consideration i the *placem nt of the electrodes* The levator and tensor muscles are located in close proximity in the si l wall of the epipharynx and in nor mal sul ject thi area cannot be observed during the insertion of the electrodes. In hi preliminary report on thi study in 1963, the author stated that he could not lemonstrate any definite differ nce between levator and tensor activity in speech With the later development and use of the piano wire electrodes and the procedures for the insertion of these however it became clear that thi preliminary finding was incorrect There i a marked difference between the levator and tensor activities in speech, and this wa also pointed out l y Bi lime *et al* (1966) It seems reasonal le to conclude that the recordings u on which the report in 19(3 was based, were both from the levators.

Various way were tried to make certain that the lectrodes were correctly inserted into the epipharyngeal wall in th position that were intended for them. The indirect epipharyngoscopy which wa carried out routinely but not always successfully d d not produce any evidence that could be used to di tingui h between levator and tensor insertions however Nor did inspection of the area l y means of an epipharyngoscope passed through the nose a ld anything f value

The most promising procedure seemed to be to stimulat the muscle through the nserted lectro le and observ the palatal response through the mouth In this way confirmation of the correct placement of the levator electrode could sometimes be btained but stimulation did not always produce a noticeable contraction of the levator muscle even though the electrode seemed to be ideally positioned and the electromyographic ignal was optimal. Never wa any kind of palatal response observed when the tensor muscle was stimulated through the piano wire electrode The reason for thi failure might have been the unfavorable position of the electrode and catheter in rela tion to the direction of the tensor muscle fibers. After the tensor catheter with a 90° angle (Fig. 3) was ma le and came into use the EMG picked up l y the tensor electrode wa alway markedly different from the typical and consistent pattern of the levator EMG however leaving no room for confusion between their electromyograms. No particular problems seemed to pertain to the indentif cation of the correct placement of the lectrodes in the superior constr ctor palatoglossus and palatopharyngeus muscles.

Another factor of importance in evaluating interf rence pattern electromyograms from muscles of this small a re is the possil ility of picking up *action potentials from adjacent muscles* by volum ond ction, a problem whicl was studied by Dedo & Dunke (1966) Th possibility cannot be left out of consideration in evaluating the data from th present tu ly Thus the tensor is bordered latero-frontally by the ptery

goid muscles. Subjects wer asked to make various jaw movements but these wer not accompanied by activity in the tensor EMG and this was taken as an indication that the pterygoid muscles did not contribute to the tensor recordi gs. However in a few recordings from the tensor muscle a background activity of low intensity and frequency synchronous with the levator activity was observed. In relation to the two types of electrodes used in this study it should be pointed out that the piano wire electrode — recording voltage across a distance of at least 2—3 mm — i more likely to be influenced by activity in adjacent muscles, than the paired fine wire electrodes — ith a very hort distance between the two bare tips. From an EMG-record made ith slow write-out speed such as the one used in this study it s very d fficult, if not impossible to distinguish between low degree of activity of close origin and activity from distant mot r units o adjacent muscles. This distinction has to be made from a record with a high write-out speed or from the oscilloscope monitor during th recording, or from a loud-speaker monitor as advocated by Shipp *et al* (1968)

In consequence the interpretation of EMG-data from a study of this type relies heavily upon the experience of the experimenter and his abil ty to cr tically evaluat the signals. As mentioned, the present invest gation wa preceded by long series of recordings d ring which the procedures were worked out W th increa ing experi nc particularly from maxillectomized subjects, the author learned to "identify" the muscles from thei typical "beha ior" fo various activities by observation f th oscilloscope screen during the placement of the electrodes.

It is not clear why sometimes no EMG-activity could be reco ded from th levator and superi r constrictor muscles. So f as the levator i concerned, it may be hy thesized that the failure was due to anatom cal ariations. The lev t r i a rel tively small muscle and fo some subjects the technique used might hav bee unfav rable resulting in an insertion latero-frontal to the muscle The failures t rec rd from the superio constrictor are more bscure This muscle ought t be a ler t [illegible] than any of the others. Sometimes epipharyngoscopy revealed th t th ground tip f the electrode h t the mucous membrane f the back wall of the pharynx at [illegible] angle with the esult that it slid along the mucous meml rane inst a [illegible] f penetrati g it

A to the normal qual ty f the peech produced d ri g the [illegible] ecordings, th subjects were t a certai tent influenced by the [illegible] and the sensation fr m the inserted electrodes. H wever the electrodes passed th ough the nose [illegible] did not interfere with rticulation, and the thin wir lectrodes inserted [illegible] usually felt only at the a gle of the mouth and most [illegible] as mentioned previously T the listener the peech prod ed by the subj t [illegible] the experiment did ot differ noticeably from the [illegible] mal peech

The close elationship between [illegible] sounds was n expected f nd [illegible] the subject. There can be no doubt [illegible] one f the muscles [illegible] f a t lity f the lev tor seemed t be more [illegible] subject than that [illegible] going from low to high [illegible] over / / to / / Also wit [illegible] the non-speech [illegible]

III A COMBINED ELECTROMYOGRAPHIC AND CINERADIOGRAPHIC STUDY ACTIVITY OF THE LEVATOR AND PALATOGLOSSUS MUSCLES IN RELATION TO VELAR MOVEMENTS

MATERIAL AND METHODS

INSTRUMENTATION

Electromyography The electromyographic equipment used in this study was the same as that described for the previous study The amplification of the EMG-signal was not kept constant however but individually set for each muscle recording to produce an optimal display on the electromyographic record. A higher paper speed was also used viz 250 mm per second

Sound recording The same sound recording system was employed as that described for the previous study The microphone was placed close to the left corner of the subject's mouth. The microphone signal was recorded on tape as well as on the EMG record Throat and nasal microphones were not used.

Cinéradiography The main components of the cineradiographic equipment made by General Electric were an x ray tube with a pulse generator an image intensifier a 16 mm movie camera, and a fluoroscopic viewer

The x ray generator was a G.E. type KXD-325 The x ray tube unit had a focal spot of 0 mm The radiation was pulsed, and the duration of each pulse was 1 msec The x ray tube factors used in this study averaged 80 KV and 1 milliampere The distance between the x ray source and the image intensifier was the same in all experiments, viz 100 cm

The image intensifier was a "Fluoricon Image Intensifier" with a diameter of 9 inches and a nominal gain of 3000, re Patterson CB 2 screen. The fluoroscopic grid had 80 lines per inch and a 6 1 grid ratio.

The camera was a General Electric 16 mm movie camera, the motor of which was synchronized with the pulsed x ray A camera speed of 60 frames per second was used The film was "Kodak Plus-X Reversal" The fluoroscopic viewer permits proper positioning of the subject before the recording, as well as observation during the recording A Scholz head clamp was used to minimize movements of the head during the recording.

Synchronization apparatus For the synchronization of the electromyographic and cinéradiographic recordings a special instrument was constructed (Fig 21) A plexiglas disc 20 cm in diameter was mounted on the axis of a small electric motor To this disc a small arrow of lead and a small bar magnet were attached. Another arrow of lead was fixed to the motor on a shaft of aluminium. Likewise a small electromagnet was fixed to the non-rotating part of the system. The arrow and the bar magnet on the disc were arranged in such a way that when the rotating arrow passed the fixed arrow the rotating magnet also passed over the electromagnet and induced a voltage which was amplified and recorded as a spike on the Visicorder (Fig 27) During the recording part of the synchronization device was placed in the radiation field close to the subject's right cheek, so that the arrows could be seen in the fluoro-

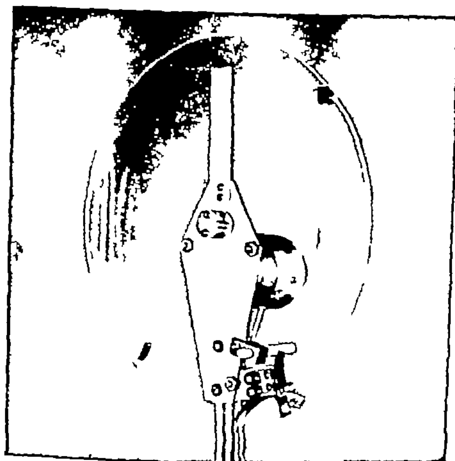


Fig. 24. The synchronization apparatus, showing the rotating plexiglas with the lead arrow and small bar magnet, the motor, the fixed arrow (vertical) and the electromagnet. It can be seen that the arrangement is such that when the rotating arrow passes the fixed arrow, the bar magnet passes over the electromagnet to induce voltage to be recorded as synchronization spike.

scopic viewer and recorded on the film (Fig. 25). The speed of the motor was approximately 2 revolutions per second, and consequently the arrows met once every 30 frames on the film.

MATERIAL

The subjects were 13 student volunteers of the University of Michigan, 2 of whom had participated in the previous study. There were 6 males and 7 females. Age r nge and dialect variation were the same as the previous study.

PROCEDURES

Recording room. The experiments were carried out in a research laboratory belonging to the Department of Radiology. The room was not sound treated. The camera noise was moderate, however, and the positioning of the microphone close to the



Fig. 45. A print of f ame from one of the cineradiographi films At the upper left the crew of the synchronization ppa tus an be seen, the rotating one less sharp than the f xed one The Eustachia tube atheter is also seen, and bov lt part f the adjust ble gl ves supporting the catheter

subject s mouth allowed for a sound recording adequate for the purposes of the study with one m nor exception a will be mentioned later in this chapter

Electrod placement Topical anethesia wa admini tered, a pianowire electrode was inserted into the levat r muscle on one side and two fine wire electrodes were inserted into one f the palatoglossus muscles in the same manner as described for the previous study

Posit oning of the subject The sul ject was seated upright in a dental chair Following the placement of th electrodes, the head clamp was applied and the head of the sul ject pos tioned to produc a straight lateral projection on the x ray film. This was done under fluoroscopic control, and so was the subsequent positioning of the synchronizing device

Recording program Tw almost identical series of sentences and isolated vowel sounds were recorded, and the xp ment was concluded by having the subject suck barium contr t uspension th gh t w and swallow Besides the aim of providing

information on the relationship between the muscle activity and velar movements in general, the speech samples were also selected to demonstrate differences in the production of low and high vowels in isolated position as well as in connected speech. The subjects read the speech samples from two series of cards, as follows

First series	*Second series*
The boot belongs to my father	The boot belongs to my father
Martin sneered at me	Martin sneered at me
Last but not least.	Least but not last.
/a/	/u/
Say sip again.	I say sip again.
/æ/	/i/
Say sim again.	I say sim again.
/i/	/æ/
Say sick again	I say sack again
/u/	/a/
Say sing again.	I say sung again.

It should be noted that in standard American English the word "last" is produced with a low front vowel /læst/ in contrast to the British English pronounciation with a low back vowel /last/

Instruction of the subject Before the experiment the subject was given a short period of training in reading from the cards Since many of the subjects were not familiar with phonetic transcription, the emphasis was placed on reading the symbols for the isolated vowels correctly and on the production of these sounds If a mistake was made by the subject during the recording, which happened a few times the item was repeated after the reading of the series

Recording. Two assistants operated the recording equipment during the experiment, while the experimenter instructed the subject and observed the oscilloscope-monitor At the beginning of the recording a specially designed plexiglas ruler with lead inlay was introduced into the oral cavity of the subject, in the midline This made the calculation of the real size of the structures possible in the following analysis of the film.

Radiation. During the experiment the subjects were exposed to radiation of approximately 0.6 rad per minute The total exposure for individual subject averaged 2 rads, with range from 1.2 to 3 rads.

Analysis of cinéradiographic films. A Kodak Analyst 16 mm movie projector was used. With this projector the film can be advanced or reversed frame by frame and kept still, or automatically projected at rates of 1 2 4 6, 8, 12, 16 or 24 frames per second in either direction. Tracings from the films were made in a way similar to the one described by Moll (1960) A special stand was built, where the projector was placed at the lower end of a sloping board. At the other, elevated end was a drawing board with glass window The film was projected through this window on to tracing paper The distance between the projector and the drawing board was kept constant and the projected image was slightly larger than life-size [illegible]

Before the analysis of [illegible] film was started every tenth frame was numbered in the left lower corner for identification purposes

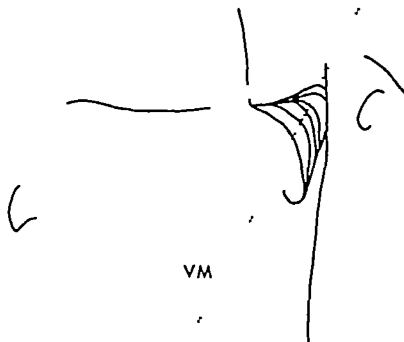


Fig. 26. Tracings f frames from el eradiographi film, superimposed to demonstrat how the VM-line was dra through the middle third f the posterior contou f the el m to represent the xi f ela movement.

The initial step in the analy is of a film was to make a preliminary record of the onset and termination of each speech sampl an l to identify by numbers those frames where movement started and ended From the appropriate locations tracings of the arrow were made for those frames where the rotating arrow most nearly coincided with the fi ed arrow of the synchronization apparatus. The temporal correspondence between the film and the EMG record could be established from these tracings of the arrows and the location of the synchronization spikes

Tracings w re made of the contours of the soft palate and the posterior pharyngeal wall, as well a of certain landmarks, primarily the frontal incisors of the upper jaw and the pterygomaxillary fissure The landmarks were used to superimpose tracings from different frames. For each subject tracings from a number of selected frames were made frames representing a series of "positions" of the palate from the physiologic rest position to "full" elevation during velopharyngeal closure These tracings were superimposed, and an bl que l ne wa drawn (Fig 26) representing the direction of movement of the elum, notably of it middle third (levator eminence) The tracing for the rest pos t on was used a a template on which each of the other tracings was superimposed All m m nt of velar movement or velar displacement presented n this study w l g th oblique line the velar movement (VM) line, from th point wh tl et ior cont ur of the soft palate crossed this line in

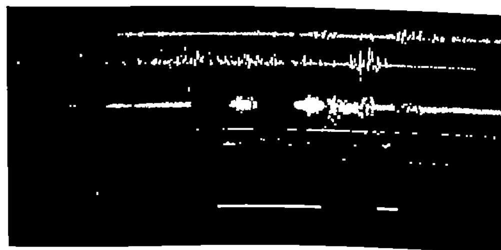


Fig. 27 A recording of the sentence, "I say sing again The measurements of elar displacement have been plotted below the Viscorder record, and elopharyngeal losure is indicated (broad white lines) The arrow shows the onset of lev tor EMG speech signal, and elar movement. Calibrations to the left of the EMG curves represent 0.5 mV There is approximately 0.5 sec between synchronization spikes.

It can be seen that velar movemen starts shortly fter the appearance f the first minimal spike in the levator EMG. There is slow increase of levator activity and slow elevation of the velum Velopharyngeal closure is not present at the onset of phonation. The palatoglossus is weakly active in the beginning. A burst of palatoglossus activity is followed losely by the descent f the velum for the nasal sounds, and burst of lev tor activity is followed losely by the elevation of the velum for the production of the f llowing oral sounds. There is marked palatoglossus activity for the final nasal sounds and quick drop of elum to low rest position before phonation has ended.

the rest position tracing This method was described and used by ...
(1967)

Eval tion f th ecords From the recordings f few ... samples were selected, and tracings were made from all ... cinéfilm Measures f ela displacement were made and plotted ... Likewise, absence presence f elopharyngeal closure was ind ... gross relationsh ps between muscle activ ity elar movement ... could be ascerta ned (Fig 27) F r the sake f simplicity ... lopes and the microphone recordings were traced nd plotted ... ment curve (Figs. 28—30) some cases

Three quant tativ analyses were carried out

1 Latency measurements for onset f levat EMG, ... gnal.

2 Latency meas rements for ncrease f EMG-activ ... descent f elum.

3 Measurements of degree of levator act ity in ... wel production.

The different measurement proced res used for the ... those sections where the result re presented.

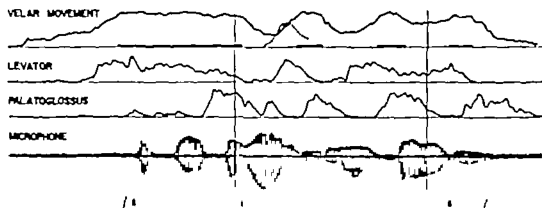


Fig. 28. \ la movement curv d lepharyngeal losure (broad black lines) plotted ainst tracings f levator d palatoglossu env lopes d microphone ignal for the sentence "The boot belongs to my f ther The ppearance of the first spik in the ra les tor EMG is indicated by n rrow over the les tor en lope \ hort segment of measu ement f the levation of the tongue for the production of /ŋ/ has been plotted on the VM curv and it relation to the pala toglossu peak corresponding to this tongue movement ca be ppreciated. The vela movement curv f llow ery losely the les tor env lope Every descent of the lum is preceded by palatoglossus acti ity \ lar displacement and les tor activity are higher for /u/ in boot tha for /a/ i father which latter sound i produced without velopharyngeal losure — The t thi ertical lines indicat the duration of 1 s.

Results

General findings

Among the 13 subjects, sati factory recordings from both muscles were obtained in 9 subject In each of the other 4 subject only one muscle recording was successful, in 2 subject that of the levator and in 2 that of the palatoglossus. Consequently the results presented below are based on data from 11 levator recordings and 11 pala toglossus recordings and on data from the corresponding cinefilms

A expected, a very close relationship between levator activity and elevation of the velum was f und (Figs 27—30) There was a striking similarity between the levator envelopes and the velar movement curves Likewise the palatoglossus activity was closely related to the descent of the velum. A fall of the velar movement curve almost always corresponded to a rise of the palatoglossus envelope

But the palatoglossus recordings also showed periods of activity which did not seem to be related to changes in the position of the velum Thus, for the production of dorso-velar sounds, /k/ /g/ /ŋ/ a corresponding burst of activity could usually be demonstrated in the palatoglossus EMG This presumably reflects palatoglossus participation in the elevation of the posterior part of the tongue (Fig 28)

Between speech samples both muscles usually showed absence of activity Before the onset of phonation both muscles were active to a varying degree and the velum was raised to a arying extent This elevation of the velum started shortly after the first minimal signs of elevat r acti ity usually before any appreciable rise of the levator envelope could be discerned (For this reason the occurrence of the first signs of the levator act ty as judged from the raw" EMG curves, has been indicated with an arrow in Figs 28—30)

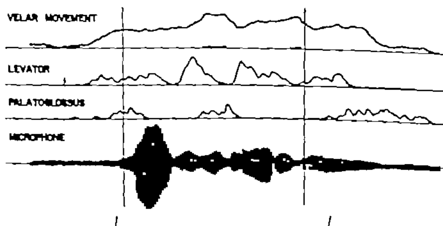


Fig. 29. Velar movement curve and velopharyngeal closure (broad black lines) plotted against tracings of levator and palatoglossus envelopes and microphone signal for the sentence "Martin sneered at me". The arrow indicates the first spike in the raw levator EMG. Levator activity and velar movement start before onset of phonation, and the velum is only partly elevated during the production of the first word. Velopharyngeal closure does not occur until the production of the s-sound, and only two brief periods of closure occurred during the production of this sentence in this subject. Palatoglossus only moderately active. — The two thin vertical lines indicate the duration of 1 s.

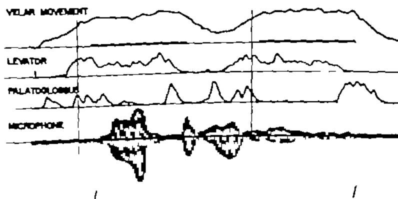


Fig. 30. Velar movement curve and velopharyngeal closure (broad black lines) plotted against tracings of the levator and palatoglossus envelopes and the microphone signal for the sentence "Last but not least". The arrow indicates the appearance of the first spike in the [illegible] levator EMG. Activity in both muscles and velopharyngeal closure before onset of phonation [illegible] EMG. Activity in both muscles and [illegible] velar movement curve follows the levator envelope. The palatoglossus muscle is active [illegible] for [illegible] descent [illegible] which is not related to velar movement [illegible] before the production of [illegible] some activity [illegible] the two *l*-sounds. — The two thin vertical lines indicate the duration of 1 s.

As demonstrated in the previous study the muscle activities precede the corresponding event in the microphone signal. In the same way the inspection of the diagrams from the present study revealed that the EMG events preceded the corresponding rise or fall of the velum (Figs 26—30).

A correspondence between the intensity of levator activity and degree of velar elevation could also be observed. This relationship was not always as conspicuous as the time relationships, but in some speech samples it was readily appreciated. Thus in Fig 28 it can be seen that the curves representing velar movement and levator activity are both higher for /u/ than for /a/. Likewise in fig 29 the levator activity is moderate in the first part of the utterance and its highest peak occurs prior to the production of the /s/; the velar movement curve shows a similar pattern, with moderate elevation in the beginning and full elevation when the /s/ is produced.

The only non-speech activities recorded in this set of experiments were sucking and swallowing. Figure 31 illustrates an EMG record on which measurements of velar movements have been plotted. Three tracings of velar "positions" have been added to illustrate the initial phases of swallowing and particularly the forward bulging of the velum, which appears to be related to a high degree of activity in the palatoglossus muscle. This indentation of the posterior contour of the soft palate was seen in 7 of the 13 subjects in this study.

LATENCY MEASUREMENTS FOR ONSET OF LEVATOR ACTIVITY, VELAR MOVEMENT AND SPEECH SIGNAL

Measurement procedures. The cinefilms of all the 11 subjects were analyzed with respect to onset of movement for each speech sample by identifying the frame in which the first change from rest position could be distinguished. This analysis was repeated a few days later. The records from these two separate determinations were then compared. In the case of the samples where the determinations were not in agreement, a third projection of the film was made and one of the two frames previously identified was selected to represent the onset of movement. The moment at which this frame was exposed was then marked on the EMG recording. The author's consistency in determining the onset of movement from the cinéfilms was calculated from the two sets of determinations made. The mean difference was 0.83 frame (14 msec) and the standard deviation 1.36 frames (23 msec).

The onset of levator activity was defined as that point, where the first minimal spike could be discerned in the electromyographic record (Fig 27).

The onset of the speech signal was defined as that point in the microphone recording where the trace emerged from the background noise. In some samples, (Fig 27) the determination of this point presented no problem, whereas in others, particularly those beginning with fricatives (/ʒ/ and /s/) the onset was obviously concealed by the noise from which the speech trace gradually emerged. Consequently these determinations did not represent the exact onset of the speech signal.

Measurements were made along the time axis to the nearest 0.5 mm of the distance from the onset of levator activity to the onset of velar movement, and this distance will be referred to as the EMG-VM latency. Likewise measurements were made from the onset of levator activity to the onset of the speech signal, and this distance will be referred to as the EMG-speech latency.

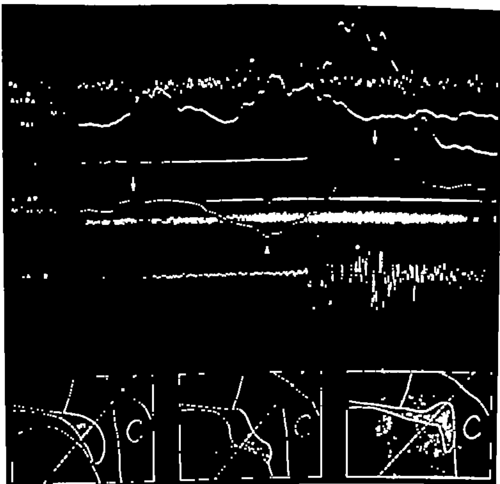


Fig. 31. A recording of swallo ring, here the elar mov ment curv has been plotted on top f the microphone and synchronization records. Trac ngs from three f mes of the y film show and thei locations ha been indicated by arrow pointing t he VM rs f III there to the lef of the EMG-curves represent 0.5 mV.
From rest position the ham is moved forward and dow rd to produc f rm seal th the tongue. An indentation can be seen in its posterior contou. This event is pparently lated to t bursts f activity in the palatoglossus muscle. From th low position he palate quickly mov to full elev tion. This movement follow losely upon strong burst of les ter acti ty.

F nd gs The mea rement f the *EMG VM latency* sh wed gr t le l f v i tion between subjects as well w th subjects. The mean was 10 ± 61 msec d the measures ra ged from —1 0 msec t 363 msec (1 10 samples out f tot l f l the VM onset preceded the EMG onset.) This id ange was due t few bad i l extreme scores, which eighted the mean. The interquartile r ge w mall r ldy smaller from 5 t 5 msec round media f 31 msec.

The measurement f the *EMG-speech latency* were f la ger may tude. The mea was 431 ± 336 msec and the total ra ge —78 t 11 1 ms (l e sampl

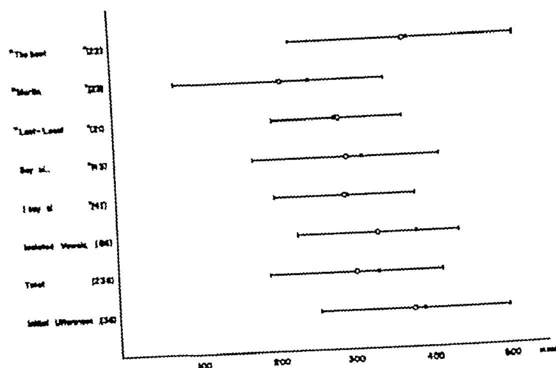


Fig. 32. Latency from onset of [illegible] activity to onset of speech signal for total set of samples and subgroups. Means (x), medians (o) and interquartile ranges plotted along the time axis. The subgroup "Martin [illegible] has [illegible] shorter latency and the subgroups "The boat [illegible] and Initial Utterances have a longer latency than the other subgroups.

beginning with "Martin [illegible] the speech signal preceded the EMG onset.) The median was 304 msec and the interquartile range 193 to 415 msec.

The total group of measurements of the EMG-speech latency was divided into subgroups with regard to the onset characteristics of the various utterances. The means for these subgroups varied from 251 to 382 msec (Fig. 32).

The subgroup beginning with "Martin [illegible] showed a mean of 251 msec and a median of 214 msec, clearly below the average. With the assumption that the samples had an approximately normal distribution, this group was tested against the total rest of samples by means of a t-test analysis (Dixon & Massey 1957). The difference was found to be significant at the 5 % level of confidence.

The cinéradiographic apparatus had an automatic switch-off system, which stopped the recording after 1 [illegible] seconds of exposure. After such a break the subject had to wait a little while until the recording apparatus had been started again, before he could resume reading. In this way the series of speech samples became "chopped up" in sets of 5—10 utterances depending upon the speed of reading. The initial utterances from each of these sets [illegible] all subjects were separated from the rest and analyzed. Their EMG-speech latency [illegible] central values well above the average of the other sub-

groups. When this group of in tial utterances was tested against the total rest of samples (t-test, Dixon & Massey 1957) the difference was f und signif cant at the 5 % level of confidence

The subgroup of utterances starting with "The boot " also had central values above the average This was ascribed to the fact that half the number of samples in this group w re initial utterances the first item on the recording program f every subject.

Summary Velar elevation began approximately 0.03—0.04 second after the first minimal spikes appeared in th levator EMG record. The onset of speech occurred approximately 0.3 second later w th wide variations. The EMG-speech latency was shorter when the utterance began with an /m/-sound and longer when the subject was waiting for a signal to start speaking.

LATENCY MEASUREMENTS FOR INCREASE OF EMG-ACTIVITY IN RELATION TO ELEVATION AND DESCENT OF VELUM

Meas ement proced res. To estimate the latency between the rise of the EMG curves and the correspond ng rise or fall of the VM curve the following procedure was used.

The onset and term nation of vela movements related to nasal sounds i medial position f selected peech samples were determined during repeated projections f appropriate portions of the cinéfilms. The "locations" of the two frames epresenting the beginning and the end of each particular movement were then marked along the time axis of the Visicorder record, and the midpoint between the two determined The EMG events corresponding to these movements were subjected to a imila analy is, i.e the lowest and highest po nts of the EMG envelopes were identif ed and the midpoint between the two was determined and projected on the time axis.

Measurements w made of the distance between the tw temporal midpoint det rmined fo EMG ncrease and elar movement, respecti ely F g 33 serves as an illustration of the method. It should be pointed out, however that for thi illustr tion measurements f velar displacement have been made fo all frames duri g th sequence and plotted as a VM curve whereas the measurements perf rmed nd presented i this section f the study were actually deri ed from determinations f the begi ing and end f mo ement only a mentioned abov

The samples f this part f the study were selected from those portions f the records which represented the fall d rise f elum within the sentences "Say im ag n "Say sing gai I say im gai " and "I say si g gai (F g 27) Fou mea rements from ea h subject pa total of 44 mea rements f ea h muscle The measurement were repeated d the means f the tw sets f mea urement wer used the analy is f the data. The author consistency in maki g these meas rement was determined from the t sets. Th d fference between the means w 0.8 ± 29 msec

F d gs The mean delay from the midpoi t f the *palatoglossu en elope rise* t th m dpoi t f *elar descent* fo im nd i g w 88 ± 32 msec When the tot l group w di ided nto tw parts, one composed f im data and the other omposed of si g data. minor difference between the means w found, but th is d fference as not stat st cally significant t test, Dixon & Massey 19)

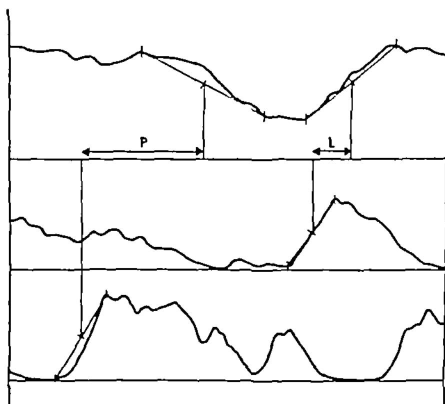


Fig. 33 Velar movement plotted against tracings of levator and palatoglossus envelopes for a short section of speech to illustrate the principles used in measuring the delay from EMG envelope to velar movement. The midpoint between onset and peak of EMG-activity were determined and the distances P and L along the time axis to the midpoint between onset and end of movements were measured

The mean delay from the midpoint of the *levator envelope rise* to the *elevation of the velum* was 12 ± 3 msec. When a division into two subgroups was made a small difference without statistical significance was found between "slm" events and "long" events

Summary The lowering of the velum for the production of nasal sounds within a sentence was preceded by increased activity in the palatoglossus muscle by a time factor of the order of 90 msec The succeeding elevation of the velum for the production of oral sounds again was preceded by increased activity in the levator muscle by a time factor of the order of 10 msec It made no difference whether a labial nasal consonant was involved or a dorso velar nasal consonant sound

DEGREE OF LEVATOR ACTIVITY IN RELATION TO VELAR DISPLACEMENT IN VOWEL PRODUCTION

Measurement procedure For the isolated vowel sounds as well as for the stressed /a/ /æ/ /i/ /u/ sounds in "The boot belongs to my father" and "Last but not least" (and the reverse) measurements were made of the degree of levator activity and the extent of velar displacement during the first 100 msec of vowel production 16 pairs of measurement were made from each subject, giving a total of 176 pairs of measures.

Act al l n g S ppl

The onset of the vowel sound was determined from the microphone record. Starting from the corresponding point on the levator EMG record, 10 measurements of the height of the rectified and averaged curve were made at 10 msec intervals along the time axis. The ¹⁰logarithm of the sum of these ten measurements was used as a relative measure of the degree of levator activity during the production of the vowel.

From the cinéfilms tracings were made of those 7 frames which corresponded to the first 100 msec of the vowel. Measurements of velar displayment were made from each of these tracings, and the mean was used as a measure of the extent of velar displacement during the production of the vowel.

Findings The means of the measurements of *levator activity* for the four vowels are presented in table 1. The means for the high vowels /i/ and /u/ were exactly the same markedly higher than the means for the low vowels /æ/ and /a/ and the means for those two were approximately the same. A comparison was also made between isolated vowels and vowels in connected speech. The difference found was very small.

The means of the measurements of *velar displacement* for the four vowels are presented in table 2. Again, a marked difference between high and low vowels was found, but only minor differences between front and back vowels. A comparison between isolated vowels and vowels in connected speech showed a difference between the means of 0.4 mm, the isolated vowels having a lower velar position.

Correlation The correlation between levator activity and velar displacement was determined by computation of the product moment correlation coefficient for each subject (16 pairs of measurements from each of them). All but 2 of the 11 correlation coefficients were significant at the 1 % level (table 3). The sample correlations ranged from 0.46 to 0.91 with an average of 0.[illegible]6 and a standard deviation of 0.11.

Analysis of variance for vowel types, positions and subjects. A factorial analysis of variance was used for testing the factors and their interactions.[1] The factors tested were A) front vowels versus back vowels B) low vowels versus high vowels C) isolated vowels versus vowels in connected speech, and D) subjects. A mixed model was used in which A, B, C were treated as fixed effects and D as random (Bennet & Franklin 1954, Ostle 1963). This analysis was carried out for levator EMG measures (see table 4) and for measurements of velar displacement (see table 5).

The results for the *levator activity* show that the difference between the means for low and high vowels was significant at the 1 % level, and the variance of the subject

TABLE 1

	Front	Back	
	/i/	/u/	
High	1.916	1.916	3.832
	/æ/	/a/	
Low	1.693	1.669	[illegible]
	3.609	3.585	

Relative measures of degree of levator activity
Means for the four vowels

TABLE [illegible]

	Front	Back	
	/i/	/u/	
High	[illegible]	[illegible]	[illegible]
	/æ/	/a/	
Low	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]10	

Measures of velar displacement (in mm)
Means for the four vowels

[1] This analysis was carried out by the Statistical Research Laboratory of the University of Michigan

DISCUSSION AND CONCLUSIONS

In this second series of experiments cineradiography was added to the electromyographic and acoustic recordings. Since simultaneous recordings could be made from two muscles only and because of the time limit set by the exposure to radiation, it was decided to confine the study to the levator and palatoglossus muscles. They were selected because of their consistent mode of action and their opposite functions in regard to palatal movement. The results will be discussed below and some aspects of the procedures and comparisons will be made with the results from studies by other authors.

The only previous study of the palate in which the same three parameters were investigated is that of Lubker (1967, 1968). He used surface electrodes held in place on the oral side of the soft palate by suction, and he ascribed the EMG activity picked up by these electrodes primarily to the levator. He used the slope of the integrated EMG curve during the production of the sound as a measure of palatal EMG activity. The production of isolated vowel sounds and /m/ and a short set of CV utterances was studied in five subjects. Repeated references will be made to Lubker's study and these basic differences between his study and the present one should be kept in mind.

The *VM line* along which the measurements of velar displacement were made (Fig. 26) was arbitrarily chosen for each subject separately. It does not represent the axis along which any particular point of the velum moves but rather the general direction of movement of the middle third of the velum. It also seems to have approximately the same direction as the fibers of the levator and palatoglossus muscles and is therefore particularly appropriate for the purposes of this study. This impression is also supported by the findings of Lubker who observed a slightly higher correlation for measurements along this oblique VM line than for measurements of velar height in the vertical plane.

The striking *similarity* between the *levator envelope* and the *velar movement curve* as demonstrated in Figs. 27—30, indicates that the relationship between the two is so close that the shape of the one can be inferred from the shape of the other. In all speech samples where sequences of frames were traced and VM measurements plotted on the EMG-records, the appearance of levator activity was accompanied by palatal elevation. Termination of levator activity was always followed by descent of the velum, and vice versa: in no instance was velar elevation observed which was not accompanied by levator activity.

The *EMG-VM latency* was short (31—40 msec). As mentioned, in 40 samples out of 236 the velar movement seemed to start before the onset of levator activity in spite of the fact that the onset of the levator activity was defined as the first minimal discernible spike in the EMG record. Most of these improbable "reversed" measurements can possibly be accounted for by the error involved in determining the onset of velar movement. The author's inconsistency in this respect was 11 ± 45 msec, as mentioned. The onset of movement may also have been caused by other muscles: the superior constrictor or palatopharyngeus muscles.

The *EMG-speech latency* findings are of the same order as those of Iwashita (1965) who studied isolated vowel sounds and monosyllables and found a mean for vowels of

270 msec and a mean for CV-syllables of 190 msec. His total range was from —200 to 500 msec. He also found that utterances starting with a nasal sound had shorter latencies than other utterances, which is in agreement with the findings of the present study. These latency measurements from the levator can also be compared to those of the laryngeal muscles, reported by Faaborg-Andersen (1957). He found an EMG-speech latency ranging from 18 to 990 msec with an average of 350—550 msec. This might indicate that the laryngeal EMG-speech latency is longer than the palatal. However, the present study has shown that the recording situation influences the EMG-speech latencies (e.g. subject waiting for signal to start speaking) and the small differences in the results of the two studies may be due to a factor of this type.

The measurements of the delay between the *increased activity in the levator* and the *rise of the velum* showed a mean of the same order as the EMG—VM latency (42 msec vs 40 msec). The average delay from *palatoglossus increase to lowering of velum* was twice as long. It can also be seen in Figs. 28—30 that lowering of the velum does usually not start until the levator activity has ceased completely or come to a standstill at a certain level, although the palatoglossus may show a great deal of activity long before that moment. This indicates that the levator is the stronger of the two and the more influential muscle as far as palatal movements are concerned.

It was first shown by Lippold (1952) and Bigland & Lippold (1954) that integrated EMG potentials vary directly with the strength of contraction in a muscle. In the present *quantitative analysis of levator activity* and *velar displacement* during *vowel production* the area under the envelope was used as a relative measure of the degree of levator activity. In a few subjects there were isolated instances of very intense levator activity out of proportion to the extent of velar displacement. To somewhat counteract the distorting effect of these very high measurements the logarithm of the area under the envelope was used in the statistical analysis of the relationships between EMG, velar displacement and vowels.

The evaluation of the VCV utterances in the previous study had indicated that in most subjects the levator was more active for /a/ than for /i/. In the present quantitative analysis of isolated vowels and vowels in connected speech, however, no difference was found. The relative measures of levator activity were the same for /a/ and /i/.

The reason for this disagreement between the two studies may be the fact that different speech samples were used. As pointed out in the preceding chapter the nasal VCV utterances demonstrated a clearer difference in levator activity than the oral utterances and in the present study none of the vowels were connected to nasal consonants. Lubker found a higher degree of palatal EMG activity for /a/ than for /i/. The measurements of velar displacement showed a clear difference between low and high vowels but minimal differences between front and back vowels, which is in agreement with the findings of Moll (1962) and Lubker (1968).

The correlation between levator activity and velar displacement varied from 0.16 to 0.91 between subjects. The reason for this somewhat wide variation is not clear, but at least two plausible explanations present themselves. The position of the electrode might have been such that a representative sample of the levator activity was not obtained, and tensor activity might have contributed to the recorded EMG signal. O

the superior constrictor palatoglossus and palatopharyngeus might play a more important role in some subjects, reducing the influence of the levator and its correlation with velar displacement. The average score of 0.76 is close to Lubker's 0.83 for isolated vowels and 0.80 for CV-syllables computed over his 5 subjects. Lubker did not report the correlation coefficients for each individual.

The analysis of variance of palatal activity during vowel production, carried out separately for levator activity and for velar displacement, gave almost exactly the same results for the two parameters. This is in agreement with Lubker's findings. Highly significant differences were found between subjects. Lubker did not report on interpersonal variations but in his "raw" data (Lubker 1967) such variations are found.

Recently Moll & Shriner (1967) presented a simple theoretical model for velar activity during speech. They hypothesized a two-mode control at the muscular level, one mode for velopharyngeal closure (oral sounds) and one mode for partial elevation (nasal sounds). The number of intermediate velar positions observed could be caused by variations in timing and the inherent restraints of the mechanical system. On the basis of the findings from his study Lubker rejected this model. The results of the present study do not support it either. The motor nerve signals to the levator muscle appear to operate along a continuum rather than in a two-mode fashion.

In conclusion, these quantitative analyses have shown that the movements of the soft palate follow very closely the activity of the levator muscle. The levator appears to have the primary control of the velum when compared to the palatoglossus muscle and, as may be inferred from the previous study, when compared to the other muscles as well. Velar movement starts within 40 msec after the onset of levator activity and speech sounds follow about 300 msec later, with wide variations, depending to a certain extent upon the initial sound and the speaking situation.

GENERAL DISCUSSION

BACKGROUND

The soft palate cannot be directly observed in normal subjects during speech, and the velopharyngeal closure mecha ism apparently remained unknown until the seventeenth century Today as a consequence of the advanced development of medical technology w are equipped with refined procedures to record and analyse movements in the velopharyngeal region An impressi e effort to study several parameters simultaneously is reported by Subtelny *et al.* (1968) They describe instrumentation and procedures for 240-frames-per-second-cineradiography synchronized with recordings of audio, intraoral and intranasal air pressure as well as oral and nasal air flow and they also outline analogue computer techniques f r additional parameters of sound power and volumetric information from sound and volume velocity recordings"

With th accumulation f data on the peripheral functi ns, li guists has directed more and more attention to th mechanisms that control the movements f the arti culators. What kind of information do the muscles recei ? Which are the motor commands for speech? How are speech sounds coded by the central nervous system? Chal lenged by these questions, speech researchers make increased use of electromyography as demonstrated in the reports by Cooper (1965) Fromkin & Ladefoged (1966) and Öhman (1967)

The discrete function of th indi idual muscles that control the movements of the palate is not altogethe clear Everyone seems t gree on the crucial importance f the lev tor and on the participatio of the superior constrictor in accomplishing elopharyngeal closur and no one ha disputed the elum-lowering function of the pala toglossus muscle Th rôle f the tensor i speech, howev is doubtful, and the contri bution of the palatopharyngeus muscl t elopharyngeal closure remains hypothetical.

From clinical point of view there is also a need fo more information on the basi mecha isms and for diagnostic tools to ascertain the function f specific muscles. I recent decades the surgical management of elophary geal incompetence has made cons derable progress, but complet restoration of the closure mechanism is still not alw obtained I primary operations for cleft palat fracturing of the hamulus f the pterygoid bone is a routine procedure What effect does this ha on tensor func tion nd on speech? Cleft palat patients with incompetent closur how great deal f mation in thei abil ty to compensate by dduction f the lateral phary geal walls, d this funct on seems to fluence the success of secondary operations such as pala t pharyngeal flap surgery To what extent ca such compens tions be trained by speech the py Ca recordings of muscle act ty be f help in evaluating the effect f such t ng

Thus from linguistic phy iological and cli ical point f iew elopharyngeal electromyography is called for Howev few EMG studies f the palatal muscles ha been published, and the alue of the nf rmation con eyed by them i limited

CONTRIBUTION OF THE PRESENT INVESTIGATION

In the present investigation, techniques have been elaborated to make intra muscular recordings from all muscles that insert into the soft palate. In order to avoid interference with articulation as much as possible a nasal approach was used for the levator tensor and superior constrictor muscles and the electrodes were inserted into the walls of the epipharynx. The only other author who has used the nasal approach successfully is Iwashita (1965). He inserted his electrodes into the soft palate however and not into the epipharyngeal walls. The very fine wire electrodes first described by Basmajian & Stecko (1962) offer great advantages in the study of mobile structures like those of the pharynx and made recordings from the palatoglossus and palatopharyngeus possible. Intra muscular recordings from these muscles have not been reported previously.

With these new techniques it has been possible to obtain gross information on the participation of each of these muscles in speech and some non-speech activities with particular attention to the differences between oral and nasal sound production. The current assumptions as to the function of the levator superior constrictor and palatoglossus muscles could be substantiated. The soft palate is actively lowered by the palatoglossus for nasal sound.

The recordings from the tensor during speech varied a great deal between subjects, in most of them there was very little activity or none at all. In those subjects where activity was recorded, no consistent mode of action was found. Thus it seems as if the tensor is of minor importance in speech production. Neither Calnan's (1953) presumption about a preparatory tensor contraction before the onset of speech, nor Wilm's (1953) theory about tensor participation in the production of nasal sound were corroborated. More research is needed however before the rôle of the tensor in speech can be considered definitely settled.

The palatopharyngeus muscle is obviously not as consistently active in the production of velopharyngeal closure as Podvinec (1952) and others postulated. The recordings of the present study made from the vertically directed fibers in the posterior pillars of the fauces always showed some degree of activity during speech, but the pattern deviated from that of the levator and there were marked variations between subjects. There is evidently no simple synergism between the two muscles. Besides its influence on the velum, it can be inferred from the findings of the present study that the palatopharyngeus muscle also assists in the narrowing of the pharynx for the production of the vowel /a/.

In the second series of experiments, the simultaneous recording of EMG, velar movement and speech sounds made possible analyses of time and intensity relationships, that had previously been studied to a very limited extent only. Gross examination of the records in which the palatal movements were plotted against the electromyograms and the microphone signal, confirmed the inferences made in the first part of the investigation concerning the close relationship between levator activity and velar elevation, and palatoglossus activity and velar descent, respectively.

The measurements of the latency between the onset of levator EMG and microphone signal showed very large variations, of the same order as those reported by Faaborg-Andersen (1957) for laryngeal EMG and Iwashita (1965) for labial, palatal and

laryngeal EMG and sound The measurements varied considerably w thin, as well as between, individuals. The cause of these large variations is not clear Iwashita (1965) found that the EMG-speech onset latencies for the levato varied with the initial sound, but he used few subjects and did not test the diff rences for statistical significance. In the present investigation it was shown that for utterances begi ni g w th a nasal sound the onset latency is short r than f other utt rances, and that waiting for a starting signal leads to a longer onset latency than speaking at will. These differences were significant at the 5 % level of confidence The wide ranges for each individual and each subgroup still remain unexplained. It is known from various detailed instrumental analyses of speech, however that t is irtually impossibl for an individual to speak a sentence in exactly the same way twice

Attempts to measure time relationships between increase of EMG-acti ity and movements of the articulators in speech do not seem t hav been made before. The findings indicated a quicker palatal response to increased levato activity than to increased palatoglossus activity This might simply reflect the fact that the levator is the larger muscle of the two. Only one type of speech sample was used for these determinations, however and the relationship might be influenced by the context.

The analysis of the relationship between degree of levator acti ty and velar elevation ga e results in agreement with those of Lubker (1967 1968) It can be concluded that th variations n elar elevation d ing speech, which also occur during prolonged velopharyngeal closure as observed by researchers using cinéradiography is determ ned by ariations n the degree of elar muscle acti ity and not by ariations in timing and movements f adjacent structures as hypothesized by Moll & Shriner (1967)

CLINICAL IMPLICATIONS

The findings of the present investigation nd cate that the tensor is of minor importance in speech. Thus, t ppears that the fracturing f the hamulus f the pterygoid bone in primary cleft palate surgery would not ha harmful consequences so far as speech is concerned

It has been demonstrated in this report that the palatoglossus muscle is ry acti in speech p oduction, with a function opposit to that f the other elopharyngeal muscles Although tonsillectomy ery seldom is ccompanied by lasti g speech pr blems t seems important that the surgeons tak great care not to damage the palatoglossus muscle duri g tonsillectomy In the present study only sub ject who still had their tonsils wer used. It would be f interest to study whether the palatopharyngeus muscle has taken over some of the palatoglossus functions in those tonsillectomized patients who ha no terior pillars left.

FUTURE RESEARCH AREA

The techniques worked ut f the present i estigation w re used i normal sub ject to explore the ma characteristics f elopharyngeal muscle acti it speech. From th base of formation, future research may depart rious d rections to specific areas of interest Such tudies may focus on parameters and detail which w not controlled or invest gated depth in the present stud g rate f speaki g.

sound intensity level, transitions between oral consonants requiring high intra-oral pressure and nasal consonants etc. It is also of interest to study subjects speaking other languages. The data presented in this report were obtained from subjects speaking American English, in which a relatively high degree of nasalization of vowels appears to be present. Is the seemingly greater articulatory effort of German speaking subjects reflected in the electromyograms? What are the differences in velopharyngeal muscle activity between oral and nasalized vowels in French?

The timing of the velopharyngeal motor events in relation to those of the larynx, tongue and lips is of considerable interest from a theoretical as well as from a clinical point of view. What is the time schedule for the speech motor commands to different parts of the vocal tract? Iwashita (1965) compared the onset of velar muscle activity with that of the orbicularis oris and lateral cricoarytenoid muscles. He found that the velar activity started later than the lateral cricoarytenoid activity for the production of vowel and voiced consonant syllables and later than the orbicularis oris for labial consonant syllables. His findings should be corroborated and supplemented by statistical analyses before clinical application is considered.

The tensor veli palatini muscle is responsible for the opening of the Eustachian tube and ventilation of the middle ear. Electromyographic recordings from the tensor with the technique described in this report would add valuable and new information when combined with tests for Eustachian tube function.

CLINICAL APPLICATIONS

Although the present study was not undertaken with direct clinical applications in mind, velopharyngeal electromyography represents a tool that can be used in clinical research and diagnosis. Velopharyngeal mistiming appears to be present in various types of velopharyngeal incompetence, particularly in dysarthria. Electromyographic analysis would add valuable data for the diagnostic evaluation of patients with these dysfunctions. Another application of direct therapeutic value would be an electromyographic assessment of the results of speech and voice therapy in patients with velopharyngeal incompetence and particularly an evaluation of the results of training on specific muscles.

SUMMARY

The investigation reported here consists f three parts. The first part is a review f the literature with an historical survey of older concepts regarding the soft palate and the development of methods t study its function, a description of the muscles of the soft palate and a report on previous electromyographic studies of the soft palate. The second part gives an account f a qualitative study of the levator and tensor eli pala tini, superior constrictor palatoglossus and palatopharyngeus muscles by mean of electromyography The third part describes combined electromyographic and ... radiographic study f th levator and palatoglossus muscles in relation to velar movements and speech, and some quantitati e analyses of these relationships.

REVIEW OF THE LITERATURE

There is no evidence that the soft palat was recognized i ancien... Middle Ages. Only references t the uvula are found. So far as we l... Vinci was the first person to describe and dep ct the soft palate in... loppio gave the first printed account of the soft palate and its musc... century is probably the era when the function of the soft palat w... In the second half of the nineteenth cent ry Czermak was plo... experiments t study the movements of the soft palate by indire... recordings of palatal movements date from this period. At the en... century x rays were first used t study the soft palat and this... important research tool n the twentieth century particularly after... the image ntensifier in the fifties. In recent decades acousti and... rch procedures hav also gi en aluable contrib tions to our kno... tion f th soft palate

The movement of the soft palat are controlled by fi pairs of m... muscle t considered.) One f these d ri es its nerv supply from th... namely the tensor eli palati i. The others re all innervated by the... to whi h the glossophary geal, agus nd accessory nerves contrib... contro s es in the l terature concerning the natomy and fun... muscle Thus the somewhat complex struct re f the tensor h... by d fferent thors and the function f the tensor i speech... disput Another issue where opinions diff rs the horizontal fi... post lo wall of the phary and inserti g int the soft palate... f anatomy usually do not recognize these fibers at all... recognize them argue about the question whether these fi... constrictor r t the palatopharyngeus. The latter muscle i a... elophary geal closure by contracting in ynchrony with... ector is posteriorly directed nd the palat horizontally...

Electromyography has become a major focus of interest for researchers in the field of speech in the last decade. Since 1958 there have been published at least 10 reports on electromyographic studies of the soft palate.

The results indicate that the overall EMG-activity recorded from the soft palate is closely related to the production of oral speech sounds and to velopharyngeal closure. Information on the discrete activity of the individual palatal muscles, however, is limited and to some extent controversial and the available data must be interpreted with great caution. The majority of the authors have used needle electrodes and an oral approach, a procedure which does not lend itself very well to the production and study of speech.

A QUALITATIVE STUDY OF THE PALATAL MUSCLES BY MEANS OF ELECTROMYOGRAPHY

Guided by a Eustachian tube catheter, a specially designed "monopolar" electrode was inserted through the nasal cavities into the tensor, levator and superior constrictor muscles respectively. Very thin wire electrodes were inserted through the mouth into the palatoglossus and palatopharyngeus muscles. Two EMG-signals at a time were fed into a modified Honeywell Electronic Medical System and recorded on a Visicorder. Rectifying and averaging circuits also provided envelopes of the EMG signals. A Du *Mont dual beam oscilloscope was used for visual monitoring of the EMG signals. Sound* was recorded on the Visicorder as well as on tape.

The subjects were 26 university students, who repeated a series of test sentences, read lists of VCV utterances and performed a number of non-speech activities involving soft palate movements.

The placement of the electrodes was difficult and not always successful. The overall rate of success was 69 %. The descriptive report is based on recordings from 10 or more subjects for each muscle.

The *levator* muscle demonstrated a close relationship to the production of oral speech sounds and hence to velopharyngeal closure. The production of nasal sounds within a sentence was preceded by decrease and disappearance of levator activity. With initial nasal sounds a low to moderate degree of levator activity preceded the speech signal. In the non-speech activities the levator was active to about the same degree as for speech. The *superior constrictor* muscle showed an EMG activity pattern of exactly the same type as the levator. The *palatoglossus muscle* showed the opposite function, closely related to the production of nasal sounds and lowering of the soft palate. The palatoglossus was also active at times which did not seem to involve velar movements, presumably participating in the elevation of the middle and posterior part of the tongue. The *palatopharyngeus* activity varied between subjects and sometimes showed very little relation to speech. When the palatopharyngeus muscle was active in speech, it seemed to be involved mainly in the production of oral speech sounds, but less consistently than the levator. The palatopharyngeus was much more active for swallowing than for any other activity. The same was true for the *tensor*, which was often completely silent during speech, and when it was active, there was very little agreement between subjects.

The placement of the electrodes is the primary consideration in the evaluation of the data. The procedures which had been developed over the years consistently gave characteristic responses from the different point of insertion, however leaving no doubt as t the source of the EMG act vity. The speech produced by the subject during the experiments d d not d ffer noticeably from their normal speech.

A COMBINED ELECTROMYOGRAPHIC AND CINÉRADIOGRAPHIC STUDY ACTIVITY OF THE LEVATOR AND PALATOGLOSSUS MUSCLES IN RELATION TO VELAR MOVEMENTS

The EMG and sound record ng equipment were the same as for the [illegible] The cinéradiographic apparatus had an ray tube with a pulse gener[illegible] image intensifier and a 16-mm movie camera. A special synchroniza[illegible] was constructed in such a w y that when a rotat ng arrow of lead was [illegible] point in the radiation field a spike was produced on the EMG rec rd.

The subjects were 13 u i ersity student who read tw almost iden[illegible] sentences and isolated vowel sounds duri g the experiment.

For the analysis of th cineradiographi films a special stand wa [illegible] image was projected onto tracing paper and traci gs were made f the [illegible] soft palate and certain landmarks from selected sequences f frames A [illegible] was drawn representing the main direction f movement f the [illegible] measurements of velar displacement were made along this a is.

When these measurements f vela displacement were pl tted on the [illegible] striking similarity was found between the levator envelopes an l the [illegible] The palatoglossus acti ity demonstrated a close elation-hip to the [illegible] as well to the production of the /k/ /g/ and n sounds In the [illegible] lowing forward bulg ng of the soft palat was seen with an indentation [illegible] rior contour n more than half of the cases. This seemed t be [illegible] contraction f the palatoglossus.

Determinat ns of onset of lev t r acti ty [illegible] la movement [illegible] were mad d the time delays measured. A me latency from acti ity t nset f ela mov ment of 10 ± 61 ms was found [illegible] from onset f lev tor cti ty t onset f sound wa [illegible] group f peech samples w divided nt subgroups t w [illegible] ing with a nasal sound /m/ had sho ter EMG speech l tency [illegible] which tiated series f peech samples [illegible] subject was w ti g for [illegible] signal t st rt [illegible]

[illegible] ttempt to est mat the dela between [illegible] f vel m withi sentence the onset nd peak p nt [illegible] en elopes a l ne dra n between these t o points and [illegible] this midpoint the time dela t the midpoint between [illegible] movement was measured The mean dela f om the [illegible] en elope rise t the midpoint f elar descent wa [illegible] f om the midpo nt of levator en lope rise to the [illegible] ment

The degree of levator activity and the extent of velar displacement was studied for four vowel sounds (/a/ /æ/ /i/ /u/) in isolated position and in connected speech.

The average correlation coefficient between the two parameters was 0.76. Analysis of variance for vowel types, positions and subjects showed statistically significant differences between low and high vowels and between subjects for both parameters. There was no significant difference between front and back vowels. Isolated vowels had a slightly smaller velar displacement than vowels in connected speech, significant at the 5 % level, but no such difference was found in the EMG measurements.

The velar movements follow the levator activity very closely. The levator muscle appears to have the primary control of the position of the soft palate. The other muscles seem only to assist the levator or to modify slightly the gross pattern of velar movements as determined by the levator.

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